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Editor: JOHN M. COULTER

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A Physiological and Chemical Study of After-Ripening — Sophia Eckerson

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BOTANICAL GAZETTE

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THE EFFECT OF CERTAIN CHLORIDES SINGLY AND COMBINED IN PAIRS ON THE ACTIVITY OF MALT DIASTASE[1]

LON A. HAWKINS

The effects produced by single inorganic salts in aqueous solution upon an organism and the modification of these effects by the presence of other salts has been the subject of considerable research. It has frequently been found that a definitely toxic influence of one salt may be completely removed by the addition of a second salt, itself also markedly toxic when used alone. This mutual counteraction by salts of the toxic influence of each other has been termed antagonism. While single salts and their combinations do not, of course, affect all organisms in the same way, yet different investigators have shown antagonistic salt action in the case of many organisms and with many chemical compounds. Thus, LOEW[2] demonstrated that the injurious action of certain concentrations of magnesium nitrate upon *Spirogyra majuscula* is inhibited by the presence in the medium of a proper amount of calcium nitrate. Similarly LOEB[3] found an antagonism to exist between the nitrates of calcium and of sodium in their influence upon the

[1] Botanical contribution from the Johns Hopkins University. No. 23.

[2] LOEW, O., Über die physiologischen Functionen der Calcium- und Magnesium-Salze im Pflanzenorganismus. Flora **75**:368–394. 1892.

[3] LOEB, J., Studies on the physiological effects of the valency and possibly the electrical charges of ions. I. The toxic and antitoxic effects of ions as a function of their valency and possibly their electrical charge. Am. Jour. Physiol. **6**:411–433. 1902.

development of the eggs of *Fundulus;* and OSTERHOUT,[4] working with plants, has shown the same kind of antagonism between the salts of magnesium and of potassium. That calcium and potassium influence each other in their effect upon certain bacteria has been brought out by LIPMAN.[5]

In a theoretical consideration of the possible causes of chemical stimulation, it is suggested that, since many vital phenomena seem to be dependent upon enzymes, it is possible that the influence of chemical substances upon the activity of plants and animals may be due in some measure to the action of such substances in accelerating or retarding enzyme activity. With these ideas in mind, it was undertaken to study the relation between the catalytic action of some enzyme and the concentration in the medium of certain salts, to determine, if possible, whether enzyme catalysis is influenced independently by two salts simultaneously present in the medium, or whether either salt increases or decreases the accelerating or retarding influence of the other upon such catalytic reactions.

The studies here reported bear upon this question and were carried out in part in the new Laboratory of Plant Physiology of the Johns Hopkins University, at Homewood, and in part in the Biological Laboratory of the Johns Hopkins University. The writer is greatly indebted to Professor BURTON E. LIVINGSTON, under whom this investigation was conducted, for his many helpful suggestions and criticisms.

Materials

The enzyme chosen for investigation was, for practical reasons, malt diastase (Merck's "diastase of malt absolute"), and the salts used were chlorides of sodium, potassium, calcium, magnesium, copper, and iron (Kahlbaum's "chemically pure" salts were exclusively used). The activity of the diastase was measured in terms of the length of time required for it to render a starch paste (of washed maize starch) powerless to give color reactions with iodine.

[4] OSTERHOUT, W. J. V., The antagonistic action of magnesium and potassium. BOT. GAZ. **45**:117–124. 1908.

[5] LIPMAN, CHARLES B., Toxic and antagonistic effects of salts as related to ammonification by *Bacillus subtilis*. BOT. GAZ. **48**:105–125. 1909.

All water used was distilled from glass and condensed in a well seasoned glass condenser. The various pieces of apparatus coming in contact with the solutions or mixtures of the experiments were carefully cleansed in this water before use.

Method

The method here employed for determining the duration of hydrolysis is similar to that described by SHERMAN, KENDALL, and CLARK as the liquefaction method for estimating diastatic activity.[6] The exact procedure in the present study was as follows: A sufficient quantity of starch for the entire study was purchased at one time and thoroughly mixed. Of this material 2.5 grams were weighed out, placed in a porcelain dish, and by means of a glass rod rubbed into a suspension with a little water. This mixture was then diluted to a volume of 500 cc., boiled two minutes, and then strained through cheesecloth, thus making a 0.5 per cent starch paste, a homogenous liquid which flows freely and is easily measured from a burette.

With the exception of a small amount used in preliminary tests, all the diastase for the investigation was purchased at one time and thoroughly mixed to insure uniformity. It was preserved in a glass-stoppered bottle and showed no signs of deterioration during the several months the study was in progress. A 2 per cent solution of this diastase was prepared in much the same manner as that employed in making the starch paste. Two grams of the dry material were first thoroughly suspended in a small amount of water and then diluted to a volume of 100 cc., after which the solution was filtered. Fresh diastase solution and also fresh starch paste were prepared at the beginning of each experiment, to preclude the possibility of error resulting from the growth of microorganisms. No antiseptic was used in the various mixtures.

Stock salt solutions, sufficient in quantity for the entire study, were prepared at the beginning of the investigation, in concentrations of 1–4 gram molecules per liter of solution, and from these the requisite dilutions were made. The iodine solution used in the tests was originally prepared in quantity sufficient for the whole

[6] SHERMAN, H. C., KENDALL, E. C., and CLARK, E. D., Studies on amylases. I. An examination of methods for the determination of diastatic power. Jour. Am. Chem. Soc. **32**:1073–1087. 1910; references to the earlier literature are given here.

research in a concentration of 1.5 grams of iodine and 3 grams of potassium iodide per liter of water.

In experimentation the various mixtures were placed in large test tubes (30 mm. in diameter and 150 mm. deep), which were suspended through the suitably perforated top of a cylindrical water-bath. The water of the bath stood always at a higher level than that of the liquid in the tubes, which were some distance apart, the outer ones being several centimeters from the walls of the bath. It was thus assured that the mixtures in the tubes were maintained at the same temperature as the surrounding water. The temperature of the bath was kept nearly constant at 50° C., by means of an ordinary mercury regulator and a gas flame below. The variations in temperature were between the limits of 48° and 52°. Since the computations of the velocity of diastatic activity were always made in terms of the velocity of the reaction in controls within the same series, the possible effects of temperature fluctuations should be exceedingly slight.

A series of mixtures was usually prepared in the following manner: In each of a series of tubes was placed the proper amount of salt solution at a concentration in each case of four times[7] the required concentration for each particular tube. To this were added 5 cc. of water, in the cases where the action of a single salt was to be tested, or an equal quantity of the solution of the other salt, in those where salt combinations were to be used; 8 cc. of starch paste (0.5 per cent) were then added to each tube, followed by 2 cc. of the previously prepared diastase solution. The tubes, containing 20 cc. of mixture, were immediately placed in the water-bath. It is thus apparent that all test mixtures contained the same quantity (0.2 per cent or 0.004 gm.) of the starch and of the diastase.

An experiment was usually made up of (1) a series of 7 different concentrations of some salt used singly; (2) a series of 7 different concentrations of another salt used singly, and (3) a series, usually in duplicate, of the 7 concentrations of the two salts in binary combinations. These with the two control tubes made a total of

[7] For 2m mixtures 10 cc. of the 4m stock salt solution were placed in each tube; this when diluted to 20 cc. by the addition of the starch and diastase gave the required concentration of the salt.

30 tubes to be tested at one time. The control tubes were prepared in exactly the same way as the others, except that water was substituted for the salt solution. In order to determine whether or not the salt solutions alone had any appreciable hydrolytic effect upon the starch, tubes were frequently made up with the salt solution and starch content the same as those in the experiment but without the diastase. These were placed in the water-bath together with the other tubes and tested from time to time in the usual manner, but in no case was there any variation from the typical blue color of the starch-iodine reaction. It was thus clear, as was to be expected, that while the salts could influence the velocity of the action of the diastase on starch, they were themselves apparently incapable of any appreciable hydrolytic action.

To determine whether the contents of any given tube had reached the end point, a single drop of the stock iodine solution was placed in each of two small vials (10 mm. in diameter and 70 mm. high). To one vial was added 1 cc. of water and to the other a like quantity of the mixture from the proper tube, carefully pipetted out. The two vials were then observed against a white background in diffused light, and if their contents appeared to be of the same color (a pale yellow) the experimental end point was considered to have been attained. It has been found that by this method of determining the end point, the color produced in a starch paste containing but 0.0002 of 1 per cent can be readily detected. This sensitiveness lies well within the limits of error of the other operations of the experiments. Final failure of one of the starch-diastase mixtures to produce color (blue, purple, red, brown, etc.) does not necessarily mean that all of the original starch content has been converted into a reducing sugar, but merely that all of the starch has been so altered as to be no longer able to form colored compounds with iodine—showing that one or several of the steps of the process of hydrolysis have been completed. In this connection it may be remarked that LANG[8] has shown with pancreatic amylase that the amount of a reducing sugar present at the time when the starch paste first fails to color with iodine does not represent and

[8] LANG, S., Über die Einwirkungen der Pankreasdiastase auf Stärkearten verschiedenen Herkunft. Zeitschr. Exper. Path. u. Therapie **8**: 279–307. 1910.

is not proportional to the original amount of starch. The present investigation is therefore not directly comparable with such investigations as those of KJELDAHL,[9] KELLERMAN,[10] MCGUIGAN,[11] and others, who measured diastatic activity by determining the amount of a reducing sugar formed in a given time. For a more complete bibliography on the effects of salts on diastatic action, the reader is referred to EFFRONT,[12] GREEN,[13] OPPENHEIMER,[14] EULER,[15] as well as to the literature cited here.

Experimentation

The water, diastase, and starch used in this work were tested for neutrality in the course of the investigation, and it was found that while the water and starch-paste were neutral to phenolphthalein, the diastase solution was strongly acid, requiring 1.2 cc. of 0.01 normal hydrate to neutralize 2 cc. of the stock solution. Thus each tube of mixture as actually used possessed an acidity equivalent to 1/1660, or 0.000602 normal. Several series were carried out by neutralizing or partially neutralizing (with sodium hydrate) this acidity of the diastase, and a strong retardation was evident when even a small quantity of the alkali was added. Thus the use of 5 cc. of 0.0005 alkali diluted to 20 cc. by the addition of the usual amounts of diastase solution, starch-paste, and water (reducing the acidity of the mixture to 0.000477 normal) caused a retardation of 13 per cent as compared with the control without the alkali. With a further decrease in the acidity to 0.000352 normal, there was apparently but little action of the diastase, and when the original acidity was completely neutralized,

[9] KJELDAHL, Recherches sur les ferments producteurs de sucre. Compt. Rend. Lab. Carlsberg **1**:109–157. 1879.

[10] KELLERMAN, KARL F., The effects of various chemical agents upon the starch-converting power of taka diastase. Bull. Torr. Bot. Club **30**:56–70. 1903.

[11] MCGUIGAN, H., The relation between the decomposition-tension of salts and their antifermentative properties. Am. Jour. Physiol. **10**:444–451. 1904.

[12] EFFRONT, JEAN, Enzymes and their applications. (PRESCOTT'S trans.) New York. 1902.

[13] GREEN, J. REYNOLDS, The soluble ferments and fermentation. Cambridge University Press. 1901.

[14] OPPENHEIMER, KARL, Die Fermente und ihre Wirkungen. Leipzig. 1910.

[15] EULER, HANS, Allgemeine Chemie der Enzyme. Wiesbaden. 1910.

no alteration in the starch could be detected by the iodine test, after a period of 5 hours in the water-bath.

A determination of the resistances of the starch and diastase solutions by means of a Wheatstone bridge and the calculation of corresponding conductivities showed the presence of a small quantity of electrolytes in the mixture. These must have modified to some extent the effect of the addition of the different salts in the experiments. Since, however, the same concentrations of starch and diastase were employed throughout the investigation, the electrolytes originally present are to be considered as constant in quantity. Furthermore, the concentrations of the salts used were usually comparatively high, so that any resulting errors due to what may be termed electrolytes of impurity (or of constitution) of the mixture used must be relatively slight.

It was observed in many cases that, in mixtures containing salts at the higher concentrations used, a flocculent precipitate was gradually formed during the experiment. This precipitate was isolated and gave no starch reaction with iodine. No relation could be detected between its occurrence or amount and the diastatic activity. It seems, therefore, that the effect of the presence of a salt in increasing or decreasing diastatic activity cannot be due to a salting out from the solution of either diastase or starch. This is in accord with the conclusion reached by COLE[16] in his work on ptyalin, but contrary to what might seem to be the case from the investigations of HARDY.[17] In this connection it is interesting to note that MUNTER[18] has recently shown that many of the salts of the alkalies and alkaline earths are ineffective for the precipitation of the diastase of *Aspergillus oryzae*.

Testing was begun when the mixtures had remained in the water-bath one hour, and was continued thereafter at intervals of 15 minutes until the conclusion of the experiment. Thirty or more

[16] COLE, S. W., Contributions to our knowledge of the action of enzymes. Part I. The influence of electrolytes on the action of amylolytic ferments. Jour. Physiol. **30**:202–220. 1903.

[17] HARDY, W. B., A preliminary investigation of the conditions which determine the stability of irreversible hydrosols. Proc. Roy. Soc. London **66**:110–125. 1900.

[18] MUNTER, F., Über Enzyme. Landwirtsch. Jahrb. Erganzband III. **39**:298–314. 1910.

similar vials were prepared before each test, each with its drop of iodine solution, and the samples were consecutively pipetted into these from the tubes, following the order in which the diastase had been originally added. The pipette was thoroughly washed after each use and thoroughly clean vials were always employed.

The number of minutes which elapsed before the end point was reached for each mixture was recorded, thus furnishing a means for the quantitative comparison of the enzyme activity under the different conditions. For example, a series of 2, 1/2, 1/8, 1/32, 1/128, 1/512, and 1/2048 molecular sodium chloride gave for the respective

TABLE I

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF SODIUM AND POTASSIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions 1:1)	
NaCl		KCl			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
2m	1.51	2m	1.77	4m	....
m/2	1.71	m/2	2.19	Mol.	1.38
m/8	1.82	m/8	1.61	m/4	1.93
m/32	1.42	m/32	1.39	m/16	1.92
m/128	0.85	m/128	0.85	m/64	1.07
m/512	0.95	m/512	0.93	m/256	0.89
m/2048	1.02	m/2048	1.00	m/1024	0.96

time periods 165, 165, 150, 180, 315, 285, and 300 minutes, the control mixture (without any added salt) reaching the end point in 285 minutes.[19] The intensity of the enzyme action in experiments such as these is, of course, for any given mixture reciprocally proportional to the number of minutes required for that mixture to reach the end point. Thus the ratio of the time period of the control to that of any mixture is obviously a measure of the velocity of the hydrolysis for that mixture in terms of the velocity of the control. Thus, the relative velocities or intensities of starch hydrolysis for the experiment just cited become, respectively, 285/165,

[19] The time periods for the two control tubes were the same in all series except three, and in these cases the two results (differing but slightly) have been averaged.

285/165, 285/150, 285/180, 285/315, 285/285, and 285/300, or 1.73, 1.73, 1.90, 1.58, 0.90, 1.00, and 0.95, when the velocity of the reaction without added salt is taken as unity.

TABLE II

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF SODIUM AND CALCIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, NaCl 2:$CaCl_2$ 1)	
NaCl		$CaCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
2m	1.51	Mol.	Retardation	3m	Retardation
m/2	1.71	m/4	1.33	3m/4	1.21
m/8	1.82	m/16	2.89	3m/16	3.29
m/32	1.42	m/64	3.69	3m/64	3.40
m/128	0.85	m/256	3.01	3m/256	2.75
m/512	0.95	m/1024	1.88	3m/1024	1.85
m/2048	1.03	m/4096	1.07	3m/4096	1.17

TABLE III

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF SODIUM AND MAGNESIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, NaCl 4:$MgCl_2$ 1)	
NaCl		$MgCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
2m	1.51	m/2	Retardation	5m/2	Retardation
m/2	1.71	m/8	2.25	5m/8	0.98
m/8	1.82	m/32	2.07	5m/32	1.90
m/32	1.42	m/128	1.97	5m/128	2.21
m/128	0.85	m/512	1.35	5m/512	1.54
m/512	0.95	m/2048	1.07	5m/2048	0.89
m/2048	1.02	m/8192	1.00	5m/8192	0.99

These numbers may be termed the ratios of the enzyme activity or of efficiency. Such a computation as the foregoing has been made in each case, always with the enzyme activity of the control for the particular experiment in question taken as unity, and it

is with the efficiency ratios for the diastase in the presence of the various concentrations of the different salts that this paper has to deal. These ratios should be comparable throughout the entire

TABLE IV

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF POTASSIUM AND CALCIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, KCl 2 : $CaCl_2$ 1)	
KCl		$CaCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
2m	1.77	Mol.	Retardation	3m	Retardation
m/2	2.19	m/4	1.33	3m/4	1.24
m/8	1.61	m/16	2.89	3m/16	2.73
m/32	1.39	m/64	3.69	3m/64	2.87
m/128	0.85	m/256	3.01	3m/256	2.87
m/512	0.93	m/1024	1.88	3m/1024	2.13
m/2048	1.00	m/4096	1.07	3m/4096	1.14

TABLE V

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF POTASSIUM AND MAGNESIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, KCl 4 : $MgCl_2$ 1)	
KCl		$MgCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
2m	1.77	m/2	Retardation	5m/2	Retardation
m/2	2.19	m/8	2.25	5m/8	1.07
m/8	1.61	m/32	2.07	5m/32	1.83
m/32	1.39	m/128	1.97	5m/128	2.07
m/128	0.85	m/512	1.35	5m/512	1.20
m/512	0.93	m/2048	1.07	5m/2048	1.12
m/2048	1.00	m/8192	1.00	5m/8192	1.08

study; where they are less than unity the salt or salts have exerted a retarding influence upon hydrolysis; where they equal unity the salt treatment has been without effect; and where they are greater than unity the effect of the salts has been to accelerate the

action of the enzyme. Of course the combination treatments possess a total salt concentration equal to the sum of the two individual concentrations of the single salts which have been combined.

TABLE VI

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF MAGNESIUM AND CALCIUM CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, $MgCl_2$ 1 : $CaCl_2$ 2)	
$MgCl_2$		$CaCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
m/2	....	Mol.	Retardation	3m/2	Retardation
m/8	2.25	m/4	1.33	3m/8	1.06
m/32	2.07	m/16	2.89	3m/32	3.06
m/128	1.97	m/64	3.69	3m/128	3.61
m/512	1.35	m/256	3.01	3m/512	2.65
m/2048	1.07	m/1024	1.88	3m/2048	2.09
m/8192	1.00	m/4096	1.07	3m/8192	1.16

TABLE VII

EFFICIENCY OF DIASTATIC ACTION AS AFFECTED BY VARIOUS CONCENTRATIONS OF FERRIC AND CUPRIC CHLORIDES, SINGLY AND COMBINED

SINGLE SALTS				COMBINATIONS (Molecular proportions, 1 : 1)	
Fe_2Cl_6		$CuCl_2$			
Concentration in mixture	Efficiency	Concentration in mixture	Efficiency	Total salt concentration in mixture	Efficiency
m/1024	Retardation	m/1024	Retardation	m/512	Retardation
m/2048	1.45	m/2048	3.50	m/1024	Retardation
m/4096	3.55	m/4096	2.62	m/2048	3.17
m/8192	3.91	m/8192	1.26	m/4096	3.59
m/16,384	1.98	m/16,384	1.00	m/8192	1.92
m/32,768	1.57	m/32,768	1.00	m/16,384	1.45
m/65,536	1.00	m/65,536	1.00	m/32,768	1.00

The total salt concentrations for the several combinations are given in the tables. It was impracticable to combine 2m NaCl with 2m KCl, so this combination was not tested. The molecular proportions at which any two salts were combined are clear from

the concentrations of the single salts standing in the same line with the datum for the combination, but these proportions are stated at the head of the columns of combinations. In those cases where the word "retardation" occurs in the efficiency column, this denotes that the efficiency here is much below that of the control without added salt, the end point not having been reached at the close of the experiment in question.

EFFECT OF SINGLE SALTS

From the foregoing tables it is apparent that all the salts used in this investigation, at some concentrations (both singly and in combination), increase the rate of hydrolysis over that of the control without added salt; at certain other concentrations they retard this process; and at still others they have apparently no influence on diastatic activity as measured in this study. Thus, with the chlorides of sodium and potassium, used singly, it may be seen that with a concentration of m/2048 the effect is practically the same as in the control with distilled water. An increase in salt content, however (to m/512), results in a slight retardation of diastatic action; while with a further increase of added salt (to m/128), the point of maximum retardation, as found in this investigation, is attained. All higher concentrations used, of these two salts, accelerate hydrolysis, the points of maximum acceleration being at a concentration of m/8 for sodium chloride and of m/2 for the potassium salt. Possibly the most striking feature of the results shown in table I is that retardation apparently occurs only at relatively low concentrations. To supplement the evidence obtained from the experiments, that there was a retardation with m/128 sodium chloride and potassium chloride as here employed, five additional series were carried out with the two salts singly at this concentration accompanied by the required controls without added salt, all made up and tested in the usual manner. In every case a marked retardation was evident in the mixtures containing the salts, as compared with the controls. This retardation cannot be due to a partial neutralization of the acidity of the diastase by small amounts of free alkali present in the salt solutions (the latter were very slightly alkaline to phenolphthalein), for table I shows that still higher concentrations of these two salt solutions, containing proportionately more alkali, unquestionably accelerate diastatic activity.

So far as the writer has been able to learn, such retardation with dilute concentrations of sodium and potassium chlorides has not been recorded heretofore. KUEBEL,[20] working with saliva, showed a slight decrease in the acceleration of hydrolysis at low concentrations of these two salts, but found no retardation such as is shown in table I, m/128 being the most dilute solution he used. This writer found a maximum acceleration between m/32 and m/128 for these two salts, and a retardation with high concentrations. He used a colorimetric method for determining the velocity of hydrolysis, comparing with tubes of colored liquids the various colors produced by the addition of iodine after a given time period. It is possible that results thus obtained may not be comparable with those of the present investigation, as the time intervals of transition between the different colored dextrins and between the last colored product formed, and achroodextrin may not be in the same proportion when the enzyme is activated by a salt as when no salt is added. Of course it must also be remembered that KUEBEL'S enzymatic mixture was saliva, while that here studied was barley diastase.

GRUTZNER,[21] using the same method of estimating diastatic activity as did KUEBEL, but working with pancreatic amylase, found the optimum concentration of sodium chloride to be between m/8 and m/32.

For dialyzed ptyalin COLE found a slight decrease in the acceleration with m/3000 sodium chloride, though acceleration at this point was still considerable. He found the point of maximum acceleration to lie between m/4 and m/300. Experiments have been carried out upon the effect of sodium chloride on diastatic action by other investigators, with varying results.

Calcium and magnesium chlorides, used singly, affect hydrolysis somewhat differently from the two monovalent salts. A decided retardation is evident at high concentrations, molecular for the calcium salt and m/2 for that of magnesium. This is in marked

[20] KUEBEL, F., Über die Einwirkung verschiedener chemischer Stoffe auf die Thätigkeit des Mundspeichels. Archiv für die gesammte Physiologie **76**: 276–305. 1899.

[21] GRUTZNER, P., Über die Einwirkung verschiedener chemischer Stoffe auf die Thätigkeit des diastatischen Pankreasfermentes. Archiv für die gesammte Physiologie **91**: 195–207. 1902.

contrast with the strong acceleration evident for sodium and potassium chlorides even at much higher concentrations. The points of maximum acceleration for calcium and magnesium chlorides, as here determined, occur at a concentration of m/64 for the former and of m/8 for the latter. From these points the acceleration falls with the gradual decrease in salt content to m/4096 calcium chloride, which accelerates but slightly, and to m/8192 magnesium chloride, which is apparently without influence. No retardation at low concentrations, as in the case of the monovalent salts, is here apparent.

The effect of the salts of the heavy metals on diastatic action (with the possible exception of mercury) have not received as much attention as those of the alkalies or the alkaline earths, which are so uniformly present in the environment of most organisms. From table VII it is apparent that the chlorides of iron and copper, as used in this investigation, retard diastatic action markedly even when present in very small quantities. Acceleration occurs in very dilute solutions, as compared with the accelerating concentrations of the other salts, the region of acceleration being found to lie between concentrations of m/2048 and m/8192 for cupric chloride, and between those of m/2048 and m/32,768 for ferric chloride, with maximum accelerations at m/2048 and m/8192, respectively. WOHLGEMUTH,[22] working with salivary diastase, found that colloidal solutions of these metals in comparatively dilute concentrations retarded diastatic action. He did not find the strong acceleration which is so apparent in the present investigation, possibly because of the fact that his metals were in colloidal condition, or because of the few concentrations that he employed. According to the data here presented, calcium chloride appears to be exceptionally effective in accelerating diastatic action, which is in accord with the findings of LISBONNE,[23] working upon the effect of this salt in restoring the activity of dialyzed salivary and pancreatic amylases in the presence of demineralized starch.

[22] WOHLGEMUTH, J., Untersuchungen über Diastasen. Biochem. Zeitschr. **9**: 10–42. 1908.

[23] LISBONNE, MARCEL, Influence des chlorures et de phosphates sur la saccharification de l'amidon déminéralisé par les amylases salivaire et pancréatique. Compt. Rend. Soc. Biol. **70**: 207–209. 1911.

In the present study with ferric chloride, at a concentration of m/4096 an acceleration was found slightly higher than any obtained with the various concentrations of copper and of calcium. It is interesting to note here the high concentrations of copper that are favorable to diastatic action, in comparison with the great toxicity of this salt toward plants as observed by KAHLENBERG and TRUE,[24] STEVENS,[25] DUGGAR,[26] LIVINGSTON,[27] and others.

A comparison of the different degrees of the acceleration of diastatic action resulting from the presence of the various salts used singly brings out the point that the effects of potassium chloride and of sodium chloride are much the same; which is in agreement with the work of KUEBEL, GRUTZNER, COLE, WOHLGEMUTH, and others, for extract of pancreas and salivary diastase. Calcium chloride, as shown in the tables, accelerates more than do the salts just mentioned and also more than does magnesium chloride. This is not in accord with the results of WOHLGEMUTH, who, however, used only a single concentration of calcium and magnesium chlorides, this being the optimum concentration for sodium chloride as he found it, a consideration which probably accounts for the discrepancy here mentioned.

EFFECT OF SALT COMBINATIONS

The average ratios of diastatic efficiency furnish a means for attacking the problem which led to the present studies, whether or not two salts simultaneously present in a mixture influence each other's effects on starch hydrolysis. While the data obtained in these experiments are not sufficiently complete to warrant quantitative consideration of this problem, several points are at least qualitatively indicated. It is apparent that, since different concentrations of the same salt produce markedly different effects

[24] KAHLENBERG, LOUIS, and TRUE, RODNEY H., On the toxic action of dissolved salts and their electrolytic dissociation. BOT. GAZ. **22**:81–124. 1896.

[25] STEVENS, F. L., The effect of aqueous solutions upon the germination of fungus spores. BOT. GAZ. **26**:377–406. 1898.

[26] DUGGAR, B. M., Physiological studies with reference to the germination of certain fungus spores. BOT. GAZ. **31**:38–66. 1901.

[27] LIVINGSTON, BURTON EDWARD, Chemical stimulation of a green alga. Bull. Torr. Bot. Club **32**:1–34. 1905.

upon the process of starch hydrolysis, any single treatment may be regarded as a combination of two separate applications of the salt in question, each at a lower concentration. The data above presented show that increasing the concentration of any salt almost never results in a proportionately increased effect upon hydrolysis. Sometimes such an increase completely reverses the direction of the effect, as when an increase in the concentration of sodium chloride from m/128 to m/32 produces an alteration in the efficiency ratio from 0.85 to 1.42. Another example of this is furnished by calcium chloride, for which a change in concentration from m/4 to molecular is accompanied by an alteration of the salt effect on diastatic action, from 33 per cent acceleration to a quantitatively undetermined, but nevertheless exceedingly marked, retardation. In most cases increased concentration of a single salt does not change the direction of the effect, but simply alters its intensity, as when an increase in the concentration of sodium chloride, from m/8 to m/2, results in decreasing the acceleration of hydrolysis from 82 to 71 per cent. It is to be expected, therefore, that the bringing of two different salt treatments into combination may frequently result in the same sort of effect as that produced by increasing the concentration of one of the single salts, the total salt concentration remaining the same as before. This is the true condition of affairs in many instances, and the specific question which confronts us—without attempting any precise quantitative comparisons—is whether combinations of two salts produce more or less effect upon starch hydrolysis than would either salt alone, at the higher concentration that obtains in the combination. Put in another way, the problem is this: if a portion of the amount of a given salt in a diastase mixture be replaced with a molecularly equal amount of another salt, is the resulting alteration in the effect upon hydrolysis merely that traceable to dilution of the first salt together with that due to addition of the second (as would be expected if the combined treatments were without effect upon each other), or is this alteration of a different character? Despite the incompleteness of the data at hand, study of the tables brings out several points bearing upon this question, which are made still more clear if graphs are resorted to. The more striking of these points will now be mentioned.

In the case of the two monovalent salts, with concentrations in the region of m/16 (where the accelerating influence of the single salts is marked, without attaining its maximum), the acceleration of diastatic action produced by combination (molecular proportions 1:1) is notably greater than that produced by either single salt. At the highest concentration used (m), however, the reverse of this proposition holds, and the combination is not as favorable to diastatic action as are the single treatments.

The four series of combinations involving sodium chloride or potassium chloride with the chloride of calcium or magnesium generally show efficiencies which follow somewhat closely those for the bivalent salt of the combination. With combinations of sodium and calcium chlorides (molecular proportions 2:1; equivalent proportions 1:1), in lower concentrations (where the calcium salt alone accelerates more markedly than does that of sodium) the efficiencies, higher than for the sodium salt alone, are not as high for the combination as for calcium chloride alone. In concentrations from 3m/64 to 3m/16 the combination results in greater acceleration than does either single treatment.

Combinations of sodium chloride and magnesium chloride (molecular proportions 4:1, equivalent proportions 2:1) show no cases where the tendency to accelerate hydrolysis is pronouncedly greater than that resulting from one of the single salts. It is best here, for the most rapid hydrolysis, to use magnesium chloride in lower concentrations (where it alone accelerates more) and to use sodium chloride in higher concentrations (where this salt accelerates more).

In concentrations of the region of m/32 the efficiencies for calcium chloride alone are markedly greater than for the combination of this salt with potassium chloride (molecular proportions 2:1; equivalent proportions 1:1), although the partial concentrations of the latter salt, when singly applied, possess a decided accelerating effect. At the concentration 3m/16, diastase efficiency for the combination used is greater than for either salt alone at concentrations nearest 3m/16.

The relations which hold for combinations of potassium chloride and magnesium chloride (molecular proportions 4:1; equivalent

proportions 2:1) are similar to those which obtain for combinations of sodium chloride with the magnesium salt. It appears that the combination is here never more favorable for starch hydrolysis than the single salt treatments.

In combinations of magnesium chloride with calcium chloride (molecular proportions 2:1) the efficiencies appear to follow those of the calcium salt, the magnesium in this combination seeming to have acquired as great efficiency for the acceleration of hydrolysis as that which characterizes the portion of the other salt which it replaces.

With combinations of copper chloride and ferric chloride (molecular proportions 1:1) the efficiencies generally lie between those for the single-salt treatments of the same concentration, as though the effectiveness of the combination were the mean influence of the two component partial treatments. No increased efficiency is here brought out by replacing a portion of one of these salts with a molecularly equal portion of the other.

While the considerations just presented appear to lead to the conclusion that certain concentrations of salt combinations may favor greater diastatic activity than do the same concentrations of either salt alone, yet it is obvious that sufficient information is not yet at hand to allow any attempt at generalization. The suggestion becomes patent that the study of properly balanced salt combinations, in their relation to enzymatic action, may add much not only to our knowledge of this persistently vague province of physiology, but also to our ability to control these important processes.

THEORETICAL CONSIDERATION OF RESULTS

In connection with the general problem of the nature of the acceleration and retardation of diastatic action by electrolytes, the results of the investigations of COLE deserve careful consideration. In a research carried on with dialyzed ptyalin, this author formulated a hypothesis to account for the influence of electrolytes on enzyme action, which to quote him directly is as follows (*op. cit.* p. 211): "The hydrolysis of starch by ptyalin is accelerated by the presence in the solution of electronegative ions (anions) other than hydroxyl ions and depressed by the presence of electro-

positive ions (kations) and by hydroxyl ions." He considers the effect of electrolytes on the rate of action to be compounded of two factors: (1) "the acceleration due to the anion; (2) the depression due to the kation." The work of this author showed that the addition of a small quantity of hydrochloric acid to a chloride in optimum concentration for diastatic hydrolysis did not result in an added acceleration, but in a decreased activity. This he accounted for by considering the acceleration produced by the chloride as due to the chlorine ions, these being already in optimum concentration before the addition of the acid; hence when more chlorine ions were added with the acid there could be no further increase in diastatic activity. The observed decrease in enzyme efficiency, which accompanied the putting in of the acid, was explained as due to the increased concentration of retarding cations which must result from the presence of the acid and to the destruction of ferment by the hydrogen ions. The salt present in a molecular condition is not taken into consideration.

This hypothesis appears to explain many of the phenomena of salt influence upon diastatic action as found in these studies. It does not, however, offer an explanation for the retardation of diastatic action by low concentrations of sodium and potassium chlorides. COLE suggests also that all cations seem to retard diastatic action alike, which would seem questionable from the standpoint of the results obtained in the present investigation.

WOHLGEMUTH, investigating the effects of certain salts on the hydrolysis of starch by pancreatic amylase, using a colorimetric method for determining the rate of hydrolysis, concludes that the acceleration caused by a chloride is due to the chlorine ion. BANG,[28] working with dialyzed ptyalin and determining the amount of a reducing dextrin formed from soluble starch in a given time, as a measure of the rate of hydrolysis, agrees with WOHLGEMUTH in this.

MCGUIGAN, working with malt diastase and using as a criterion for comparing the different salt effects the inhibition of the formation of any reducing sugar in a certain time period, takes a view opposite to that of COLE. He considers the acceleration of enzyme

[28] BANG, IVAR, Untersuchungen über Diastasen. Biochem. Zeitschr. **32**:417–442. 1911.

action brought about by a salt as due to the effect of the cation and the retardation as due to the anion. Although his results are not directly comparable to those of the present investigation, it may be noted that most of the salt effects here brought out may be explained by this theory about as satisfactorily as by that of COLE. It is manifestly impossible, however, from the evidence brought out in this investigation, to decide whether either theory is adequate to explain satisfactorily the phenomena of acceleration and retardation of diastatic action by electrolytes. It is, of course, probable that diastatic action is, in some measure, affected by the presence of salts in a molecular condition, also that certain salts form compounds with the enzyme itself, or with other organic bodies which are present in the diastatic mixtures that have usually been used in experimentation of this sort. Likewise diastases of different origins may be influenced quite differently by the same electrolytes. Further and more careful experimentation, with refinement of methods as suggested by FORD,[29] SHERMAN, KENDALL and CLARK, FRANKEL and HAMBURG,[30] and others, are still much needed.

Summary

The present investigation deals with the effects of sodium, potassium, calcium, magnesium, cupric, and ferric chlorides, alone and in certain binary combinations, on the hydrolytic activity of Merck's "diastase of malt absolute," the enzymatic mixture acting on a boiled solution of washed maize starch, at 50° C. The disappearance of the ability of the starch to give a color reaction with iodine was taken as the end point of the reaction, and the reciprocal of the time period which elapsed before this end point was attained (considering the time period of the control without added salt as unity) was used as a measure of the intensity of enzyme action.

A wide variation is clearly shown in the influence of the different chlorides upon diastatic action, which is probably to be related to the properties of the various cations employed. More or less

[29] FORD, JOHN S., Lintner's soluble starch and the estimation of diastatic power. Jour. Soc. Chem. Ind. **23**:414–422. 1904.

[30] FRANKEL and HAMBURG, Über Diastase. Beitr. Chem. Physiol. u. Path. **8**:389–398. 1906.

pronounced acceleration of starch hydrolysis is shown for all of the salts used at different concentrations; the highest acceleration found is for iron (291 per cent) and the next highest for calcium (269 per cent). Retardation of hydrolysis is shown at high concentrations for all salts excepting sodium chloride and potassium chloride. For these two salts a pronounced retarding action (15 per cent in both cases) is manifest at low concentrations, the greatest retardation occurring with concentration m/128. This retardation in weak solution seems not to have been considered heretofore.

Combinations of two salts are shown to be sometimes more and sometimes less efficient in modifying diastatic action than are molecularly equal concentrations of their component salts. It is thus possible that enzymatic power, the magnitude of which is frequently to be related to the concentration of single salts in the medium, may in some cases at least be still more highly developed than is possible through the influence of single salts, by the presence of a properly balanced salt combination.

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A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 170

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Many seeds and spores require a long time for germination. The term "after-ripening" has come into rather general use to designate the changes in the seed during this period. It is often loosely used to include disintegration of the seed coats as well as protoplasmic or metabolic changes in the embryo. It seems better to limit its use, as has been done in this laboratory, to those cases where the delay is due to characters of the embryo. In the majority of seeds thus far investigated, the delayed germination is due to the exclusion of water or of oxygen by the seed coats. A few seeds have been studied, however, which do not grow when all coats have been removed and the embryo put in good germinating conditions. Some change within the embryo is necessary before germination, that is, lengthening of the hypocotyl, can take place. This process is what we mean by "after-ripening."

NOBBE and HANLEIN (**45**), WIESNER (**52**), JOST (**29**), and others assume, in cases where water enters the seed coat, that growth after a long period is due to some change going on within the embryo during the seemingly dormant period. This has been determined definitely for only three or four species.

LAKON (**35**) finds that the delayed (1–2 years) germination of *Pinus silvestris*, *P. Strobus*, and *P. Cembra* is not due to coat characters. With the coats broken or removed, the time required for germination was not shortened. Seeds of *Fraxinus excelsior* (**36**) sown in the spring do not germinate until the following spring. In the mature seed the embryo occupies about half the space within the endosperm; the rest is occupied by a mucilaginous substance. During the year that the seed lies in the ground, the embryo grows in length and fills the seed coat. Since the embryo is fully mature at maturity of the seed, but a period of growth is

necessary before germination, LAKON calls this "Vorkeimung" instead of "Nachreife."

Many methods have been used by different workers in the attempt to shorten the resting period of buds and bulbs. The first of these is JOHANNSEN'S (**30**) treatment with ether. He found that growth could be hastened at the beginning of and near the end of the resting period, but not in the middle period. MOLISCH (**40**) immersed shoots in water at 35° C., and the buds opened earlier than those on untreated shoots. IRAKLIONOW (**23**) finds that the warm bath increases respiration only in the first days, then the respiration curve falls to its original height. MÜLLER-THURGAU and SCHNEIDER-ORELLI (**42, 43**) used the warm bath method to hasten the germination of potato tubers, and lily of the valley bulbs. They find the sugar content increased by the warm bath, but this is immediately used by the increased respiration. Unless the bulbs are kept at a high temperature, there is no lasting effect. Zero temperature increases the sugar content and the respiration; injury produces a slight increase in sugar content. They do not believe that hastened growth in these cases is due to increased sugar content, but rather that the high temperature has some effect on the protoplasm. CHRISTENSEN (**3**) made chemical analyses of resting and growing bulbs, but found no appreciable differences. He concluded that the slow growth of resting bulbs was not due to lack of soluble food materials.

WEBER (**53**) and JESENKO (**27**) found that injury to the buds hastens their development. Later, JESENKO (**28**) found that the shoots from these buds were abnormal. Shoots immersed in dilute solutions of alcohol, H_2SO_4, and other substances, develop normally and more rapidly than untreated shoots. LAKON (**37**) forced the development of winter buds by standing the cut ends of shoots in Knop's solution. MOLISCH (**41**) has recently found radium emanations effective.

All these various methods shorten somewhat the rest period of bulbs and winter buds. Little has been done to determine what is the limiting factor to growth in these cases and what internal change is produced by the external application.

CROCKER (**4, 5**) found that the long period required for germina-

tion of seeds of hawthorn is due in part to seed coat characters and in part to characters of the embryo. With testas removed, in light at room temperature, the cotyledons increase greatly in size and turn green, but only a small percentage (2–5 per cent) of the hypocotyls grow.

DAVIS and ROSE (**6**), working in this laboratory, studied further the germination of seeds of *Crataegus mollis*. They find that under ordinary conditions seeds with carpels intact require one year or more for germination. Embryos with carpels removed, but with testas intact, germinated after 90–96 days at 5–6° C.; 74 per cent germinated after 75 days in the cold when removed to the warm greenhouse. With both carpels and testas removed, after 28 days at 6° C. 78 per cent germinated in 5 days in the greenhouse. Thus with all coats removed there is still a delayed germination due to characters of the embryo itself, a period of "after-ripening" necessary before elongation of the hypocotyl can take place. It has been my purpose to study the changes within the embryo during this period.

Investigation

I have made a preliminary microchemical study of the chemical changes during after-ripening. These results form the basis for a quantitative study. This paper gives the results of the microchemical study, together with quantitative determinations of the substances in the embryo at different periods during after-ripening.

DAVIS and ROSE (**6**) found the best conditions for after-ripening to be a temperature of 5–6° C. This inhibits growth of the cotyledons and is favorable for the metabolic changes within the hypocotyl. I studied the after-ripening of several species of *Crataegus*.

Seeds with testas intact were soaked 16 hours at 5° C., were washed thoroughly by shaking in a bottle of distilled water (to prevent mold), and put in dishes on moist cotton in the ice chest at 5–6° C. Microchemical tests were made once a week during the after-ripening period. Sections were preserved in glycerin for comparison.

MICROCHEMICAL METHOD

In order to detect the metabolic substances of the cell as nearly unchanged as possible, observations must be made on the living tissue. Sections were made on a freezing microtome or free-hand, and *intra-vitam* stains (**8**) were used. Of the different methods and stains used, the following were found to be the most valuable.

Fats.—Soudan III or Scharlach R, dissolved in 50 per cent alcohol. All fats are soluble in these stains.

Lecithin.—The fats were first dissolved out with acetone, in which lecithin is not soluble. It was then stained with Soudan III, or blackened with fumes of osmic acid.

Starch.—The sections were first heated in water on the slide, and then a drop of iodine solution added.

Sugar.—For the reducing sugars Fehling's solution was used as follows. The sections were heated in the copper sulphate solution, on the slide. The other part of Fehling's solution, Rochelle salt and sodium hydroxide, was then added. Copper oxide is deposited in the cells containing sugar. Still better is the osazone test as modified by MANGHAM (**38**). Dissolve phenylhydrazine hydrochloride and sodium acetate in 10 times their weight of glycerin; warm to dissolve; filter once or twice. To use, put a drop of each on the slide, mix, put section of tissue in same. Place slide in warm oven 5–30 minutes, and then cool. Osazone crystals will be formed in the cells which contained sugar.

Acidity or alkalinity.—Neutral red, sulphate of Nilè blue, Dahlia violet, and methyl orange. A 1/5000 solution of neutral red is sensitive to N/11,000 NaOH; a 1/5000 solution of sulphate of Nile blue is sensitive to N/5100 NaOH; the others are less sensitive (**9**).

Catalase.—A drop of H_2O_2 was put on the section on the slide and evolution of oxygen noted.

Oxidase.—A few drops of freshly made solution of guaiaconic acid, on the slide.

Peroxidase.—A drop of H_2O_2 added to a few drops of guaiaconic acid, put on the sections on the slide.

TABLE I

CONDITION OF THE EMBRYO OF CRATAEGUS GLORIOSA

Glucoside amygdalin	Condition of seeds	Fatty oil	Lecithin	Protein	Sugar	Starch	Catalase	Peroxidase	Oxidase	Acidity
HCN.....	Air-dry	Abundant (25 to 30 per cent)	x	x	o	o	x	Hyp. little Cot. x	o	Hyp. basic Cot. acid
	After-ripened	Slight decrease	x	x	Trace	o	Slight increase	Great increase	x	Great increase
	At germination	Decrease	x	x	x	x	x	x	x	x

The value of neutral red as an indicator has been questioned by many. If the change in color of a dye is due to H ion content, it can safely be used as an indicator. FRIEDENTHAL (**13**) gives a very valuable series of indicators with the H or OH ion concentration which will produce a change in color. In the presence of substances which form combinations with the dye, the change in color would be no indication of H or OH ions. In all my work, however, the acidity or alkalinity as shown by neutral red was confirmed by titrations with NaOH or HCl.

Microchemical tests should be followed always by quantitative determinations at the critical points. When so used the method is reliable and is a great saving of time and material.

RESULT OF MICHROCHEMICAL TESTS

The condition of the embryo at the beginning and end of the after-ripening period, and at germination just after the hypocotyl has pushed out through the testa, is shown in the accompanying table (I). In all the tables, *x* indicates presence in quantity, and o indicates absence.

There is a very gradual, though constant, increase in the acidity and in the enzymes during the whole period. After 80–90 days at 5° C.,

when the acidity has almost reached its maximum, the fats begin to break up and sugar appears. Oxidase first appears at this time. Hydrocyanic acid appears after 75 days, increases up to germination, then decreases (**19, 49**).

WATER-HOLDING POWER

Since the hypocotyls of the air-dry seeds are basic, while the cotyledons are acid, it was thought that they might have less ability to take up water than the cotyledons. To test this, seeds were taken at different stages of after-ripening, the coats removed, and the naked embryos soaked in water for 5 hours. These were then dried carefully with filter paper and, after the hypocotyls were separated from the cotyledons, were put in separate weighing bottles. These were weighed, then heated in an oven 10 minutes at 100° C., removed from the oven, and dried at 50° C. Many determinations were made. The accompanying table (II) gives a typical series.

TABLE II

WATER-HOLDING POWER OF EMBRYO OF CRATAEGUS GLORIOSA

	Hypocotyls			Cotyledons		
	Wet weight grams	Dry weight grams	Percentage water	Wet weight grams	Dry weight grams	Percentage water
Coats removed and soaked 5 hrs.	0.0157	0.0121	23	0.2690	0.1542	42
90 days at 5° C.	0.0189	0.0116	38	0.2700	0.1744	35
Germinated (hyp. 3 mm.)	0.0289	0.0115	60	0.2094	0.1260	39

ACIDITY

Determinations of acidity were made at the same periods of after-ripening as the water determinations (table III). The embryos were washed in distilled water which had been boiled to expel CO_2.

It will be seen that the metabolism of the fats does not begin until the acidity has reached a certain amount, the water content has increased greatly, and the enzymes are set free. The hypocotyl does not elongate until that time. It has long been known that

considerable free fatty acid is formed during the germination of oily seeds (GREEN **17**, SCHMIDT **50**). MILLER (**39**) finds, in the germination of *Helianthus annuus*, that the quantity of free acid in the hypocotyl increases rapidly at the beginning of germination.

IVANOW (**24, 25, 26**) studied the metabolism of fats in ripening and in germinating seeds. He finds that the rapidity of oil transformation in germinating seeds depends on the fatty acid components of that particular oil. The oil of flax and of hemp contains

TABLE III

ACIDITY OF EMBRYO OF CRATAEGUS GLORIOSA

	Hypocotyls			Cotyledons			
	Grams	N/20 KOH cc.	N/20 KOH cc. per gram		Grams	N/20 KOH cc.	N/20 KOH cc. per gram
Air-dry	0.0267	Slightly basic	0.00	Air-dry	0.5374	0.20	0.37
30 days	.....	.....	0.10	30 days	.....	....	0.40
60 days	.....	.....	0.30	.....	.....	....	....
90 days at 5° C. (hyp. 2 mm.)	0.0444	0.04	0.90	Cots. white	0.8162	0.35	0.42
Germinated (hyp. 2.5 cm.)	0.360	0.45	1.24	Cots. yellow	0.1914	0.60	3.13
Germinated (hyp. 3.5–5 cm.)	0.5967	0.65	1.09	Cots. green (in light 1 week)	0.3907	1.20	3.07
Germinated (hyp. 3–5 cm.)	0.3810	0.60	1.57	.....	0.7099	1.90	2.67

the less saturated acids—linolenic and linoleic—and is transformed much more quickly than that of rape, which contains oleic acid. The less saturated acids disappear rapidly (forming carbohydrates) from the seedlings and cause the very low acid number of flax and hemp. Unsaturated acids of the oleic acid type are more stable and inactive; therefore rape shows a higher acid number than flax or hemp. The acids in the seeds of *Crataegus* were not identified; this will be done later.

DELEANO (**7**) studied the chemical changes during the germination of *Ricinus communis*. He finds that the acidity and the

catalase increase up to a maximum, which is reached on the eighth day of germination (the hypocotyl is then 2.5 cm. long). Then the fats begin to break up and within two or three days disappear. The fats are transformed into a soluble substance of the character of a plant mucilage. This is later transformed into sugar, cellulose, and other substances. DELEANO says that the acids activating hydrolysis are formed during germination; he detected acetic and lactic acids. He thinks that catalase is directly concerned with hydrolysis of the fats. This is doubtful, however, since catalase is so universally present. Peroxidase reached a maximum about the fourteenth day of germination (the hypocotyls were 8.5 cm.).

The chemical changes during the 90 days of after-ripening of *Crataegus* are the same as those of the first 8 days of germination of *Ricinus*. It is as though the chemical processes, telescoped in *Ricinus*, are drawn out in *Crataegus*.

Seeds of the crab apple (*Pyrus baccata*) after-ripen in 30 days at 5° C. At the beginning of their after-ripening period, hypocotyls of these embryos have an acidity and a water-holding power slightly greater than those of *Crataegus* after 60 days at 5° C. (hypocotyl 1.45 cc., N/20 KOH, moisture 48 per cent; cotyledons 0.368 cc., moisture 39 per cent). Peroxidase increases gradually from a very little in the air-dry seeds to a considerable amount at germination. As in *Crataegus*, oxidase does not appear in hypocotyls of *Pyrus baccata* until immediately before germination.

EFFECT OF ACIDS

FISCHER (11) finds that when seeds of water plants (*Alisma*, *Sagittaria*, and *Sparganium*) are treated with dilute solutions of acids, or the strong alkalies KOH and NaOH, the percentage of germination is increased. He conceives the H ions of the acids, and the OH ions of the alkalies as destroying the equilibrium of the cell and starting up the chemical processes.[1] Seeds treated with solutions of the fatty acids (formic, acetic, propionic, and butyric) did not germinate. He therefore considers these acids toxic; but he used too concentrated solutions. In dilute solutions the

[1] CROCKER has found that these seeds germinate readily if the coats are broken or removed. The protoplasm is not dormant.

fatty acids shorten the after-ripening period of both hawthorn and apple.

Mlle. PROMSY also used acetic acid with good results on seedlings of tomato and corn. She studied (**47, 48**) the effect of acids on the respiration of germinating seeds of tomato, corn, barley, *Dioscorea*, and *Elaeis guineensis*. She finds that organic acids (citric, malic, oxalic, tartaric, and acetic) increase the respiratory quotient and at the same time the intensity of respiration, measured by evolution of CO_2. Inorganic acids, on the other hand, do not modify the quotient, except in the single case of the fatty seeds of *Elaeis*. Seeds were soaked in a solution of the acid for 24–48 hours, then put in germinative conditions in sand, and the sand watered with the solution. Seedlings submitted to the action of organic acids grew more rapidly than the control, increased more in wet weight, and increased more in dry weight, if determined at the end of the germination period when the plants were green. Seeds treated with inorganic acids, HCl and H_2SO_4, germinate more quickly than the control. The wet weight of seedlings is increased, dry weight is the same as the control.

MARTIN FISCHER (**12**) found that acids greatly increase the absorption of water by colloids, while salts decrease the absorption. He studied the absorption of water by gelatin, fibrin, and frog muscle.

A certain degree of acidity seems to be necessary before germination of *Crataegus* seeds. The acidity of the hypocotyl develops very slowly and little water is absorbed in the early stages of after-ripening. It was thought that absence of free acids might be the limiting factor to growth. An attempt was made to supply this by soaking the seeds in acid before putting them in after-ripening conditions.

METHOD OF TREATMENT WITH ACIDS

Seeds of *Crataegus*, with carpels removed but testas intact, were soaked in the acid solution over night at 5° C., washed, and put on moist cotton in Petri dishes. At the end of 14 days they were washed carefully, the coats removed, and the embryos washed in distilled water. They were then tested for presence of free enzymes. In the accompanying table (IV) the results are given

in order of the depth of color of the reaction for peroxidase and oxidase.

TABLE IV

ENZYMES IN HYPOCOTYLS OF CRATAEGUS DUROBRIVENSIS

	Catalase	Peroxidase	Oxidase
Air-dry at 5° C. 14 days	Very little	Very pale	o
Control	Little	Light blue	o
N/1000 HCl	x	Light blue	o
N/100 acetic	x	Slightly darker	o
N/50 butyric	x	Slightly darker	?
N/70 butyric	x	Darker	Trace
N/3400 HCl	x	Darker	Trace
N/1000 acetic	x	Like after-ripened	Dark blue
After-ripened 90 days at 5° C.	x	Dark blue	Dark blue
Germinated	x	Deep blue	Darker

In 14 days embryos treated with N/1000 acetic acid attain an enzyme reaction equal to that of the untreated embryo after 90 days' after-ripening. Both N/3400 HCl and N/70 butyric show a great increase in enzymes over the control.

The acidity was determined in several cases. The testas and embryos were always carefully washed in boiled distilled water to prevent any trace of external acid vitiating the results. The accompanying table (V) gives the results of one series.

TABLE V

ACIDITY IN EMBRYOS OF CRATAEGUS DUROBRIVENSIS

IN ACID OVER NIGHT	HYPOCOTYLS			COTYLEDONS		
	Grams	N/20 KOH cc.	N/20 KOH cc. per gram	Grams	N/20 KOH cc.	N/20 KOH cc. per gram
Control	.....	Slightly basic	....	.....	....	0.37
N/70 butyric 16 hours	0.0184	Neutral	0.00	0.3544	0.16	0.45
N/70 butyric 14 days at 5° C.	0.009	0.01	1.11	0.3138	0.65	2.06
N/100 acetic 14 days at 5° C.	0.0119	0.02	1.68	0.2420	0.40	1.65

After seeds have been in N/70 butyric acid 16 hours, the outer cells of the cortex of the hypocotyl give an acid reaction with Dahlia

violet. The inner cells are still basic. This shows that the acid had penetrated a little way. The inner cells develop acidity much more rapidly than in the control.

Of seeds treated as above with N/70 butyric acid, and after-ripened at 5° C. with testas on, 52 per cent germinated in 53 days; the others decayed. The testas were removed from a lot of seeds after soaking in N/70 butyric acid, and the naked embryos kept on moist cotton at 5° C.; these germinated in 16 days. As shown by the large number of seeds which were killed, N/70 butyric is too strong. More dilute solutions are being used now.

N/800 butyric has about the conductivity of N/1000 acetic and N/3400 HCl, which were found to be effective; therefore that is being tested as well as still more dilute solutions. The more dilute solutions are not toxic, instead they greatly increase the rate of the process of after-ripening; therefore, germination is hastened. The after-ripening period of seeds with testas intact was shortened from 80–90 days to 45–53 days; with testas removed, from 30 days to 16–18 days.

Summary

Condition of the embryo in dry storage.—Food is stored in the embryo in the form of fatty oil; there is also considerable lecithin; neither starch nor sugar is present. The reaction of the cotyledons is acid, but the hypocotyl is slightly basic. The water-absorbing power of the hypocotyl is less than 25 per cent of the wet weight.

There is a series of metabolic changes in the embryo during the period of after-ripening. The initial change seems to be an increased acidity. Correlated with this is an increased water-holding power, and an increase in the activity of catalase and peroxidase.

Near the end of the period of after-ripening there is a sudden increase in the acidity, and in the water content; here oxidase first appears. All of these increase until the hypocotyl is 3–5 cm. long. At this time the fats decrease and sugar appears. Hydrocyanic acid is present in the cotyledons.

The after-ripening period can be greatly shortened by treating the embryos with dilute acids, HCl, butyric, and acetic. The water-holding power, the acidity, and the amount of peroxidase increase much more rapidly, and oxidase appears much earlier, than in untreated embryos.

It is evident that there is a correlation between acidity of the hypocotyl of *Crataegus*, its water-absorbing power, production of enzymes, and germinating power. Whether the acidity is causal or merely correlative is not known. There is some evidence, however, that it is causal. GREEN (**17, 18**) has shown that it leads to the liberation of enzymes; and MARTIN FISCHER (**12**) that it increases the water-absorbing power of colloids. Other dormant seeds of the Rosaceae are now being studied with the hope of gaining further knowledge on this point.

Acknowledgments are due Dr. WILLIAM CROCKER, under whose direction the work was done.

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THE CALIFORNIA PAROSELAS

S. B. PARISH

(WITH FIVE FIGURES)

This paper is based on a study of the ample collections in the herbarium of the University of California, and of the material in the private herbaria of Dr. A. DAVIDSON and of the writer. While the numerous extralimital material in these collections has been carefully studied, the citation of specimens is confined, for the most part, to those collected within the boundaries of the state. Dr. HALL not only placed the collections of the University at the writer's disposal, but allowed him the use of a preliminary study which he had prepared, and most generously aided him in other ways. For the drawings, from which the figures have been reproduced, the writer is under obligations to the skillful pen of Mrs. CHARLOTTE M. WILDER.

The generic name

The genus *Dalea* was founded by LINNAEUS in 1737, in his *Hortus Cliffortianus*, but in the *Species Plantarum* of 1753 he reduced it to *Psoralea*. The latter year being now accepted as the initial date for phanerogamic nomenclature, we are debarred from going back to LINNAEUS' earlier use of the name. It was revived by JUSSIEU in 1789, and according to present rules that must be taken as the authoritative date for the name, and it is with this citation that it is maintained in the new GRAY'S *Manual*.[1] Unfortunately, in the interval it had been used for two other genera. In 1756 P. BROWN applied it to what is now universally regarded as *Eupatorium*, so that, as a synonym, his use may be disregarded. But GAERTNER, in 1788, gave it to what ENGLER and PRANTL and the *Kew Index* now call *Microdon* Choisy (1823). This scrophulariaceous genus, therefore, has the prior title by a single year, and the leguminous genus must take the later name *Parosela* Cav. (1802).

[1] In the *Natürlichen Pflanzenfamilien*, *Dalea* Linn. is retained.

This needless and confusing change should have been provided against in the list of *nomina conservanda*, and it is to be hoped that the oversight may be remedied in a future revision.

Distribution

Mexico is the center of distribution of the genus, fully 120 species (119, CONZATTI) having been described from the temperate regions of that republic. Thence it extends along the Andes to Chile, where it has its southernmost representative in *Dalea multifoliata*, at 30° south latitude. Crossing the United States' boundary, it is well developed in the Lower Sonoran life-area; 17 species are found in Texas (COULTER), 19 in New Mexico (HAMMOND), and 33 in Arizona (THORNBER). The most northern species is *Parosela alopecuroides*, which reaches southeastern North Dakota, at latitude 30° N., and the same species extends east to Tennessee (GATTINGER). Besides the continental species, two outlying ones are found on the Galapagos Islands. In California it is a characteristic genus of the deserts, a single species passing into an arid border of the cismontane region.

Parosela

PAROSELA Cav. Descr. 185. 1802.—*Dalea* Juss., Gen. 355. 1789; *Asagraea* Baillon, Adansonia **9**:232. 1870.—Annual or perennial herbs, shrubs, or trees, more or less glandular-dotted. Leaves odd-pinnate, rarely simple, with minute subulate stipules. Flowers in spikes or simple racemes, rarely scattered or solitary; bracts caducous, in ours subulate and inconspicuous; calyx 5-toothed; petals all with claws, that of the usually cordate banner inserted at the bottom of the calyx, and those of the wings and keel adnate below the middle of the cleft stamineal sheath; stamens 10, rarely 9, monadelphous; anthers uniform; ovules 2, rarely 4–6; pod membranaceous or chartaceous, indehiscent, 1-seeded.

ARTIFICIAL KEY TO THE CALIFORNIAN SPECIES

Perennial herbs.

Calyx long-villous, its teeth filiform.....1. *P. mollis.*

Calyx silky-canescent, its teeth ovate-acute..2. *P. Parryi.*

Woody shrubs.
Flowers in condensed headlike spikes.
Stems conspicuously guttate-glandular; leaflets 1–5 mm. long.....3. *P. polyadenia.*
Stems sparsely prickle-glandular; leaflets 1–2 cm. long.....4. *P. Emoryi.*
Flowers in loose spikes or racemes.
Leaves pinnate, or a few simple.
Leaves and twigs hoary-tomentose.....5. *P. neglecta.*
Leaves and twigs appressed silky-pubescent.
Leaflets distinct.....6. *P. Johnsonii.*
Leaflets decurrent or confluent.....7. *P. californica.*
Leaves mostly simple, but a few 3-pinnate..7a. *P. californica simplifolia.*
Leaves all simple, glabrate .....8. *P. Schottii.*
Spinose tree, hoary-pubescent, nearly leafless...9. *P. spinosa.*

SYNOPSIS OF THE SPECIES

* *Ovules 2; pod included*
† *Flowers spicate; herbs*

1. PAROSELA MOLLIS (Benth.) Heller, Cat. N. Am. Pl. ed. 2. 6. 1900.—*Dalea mollis* Benth. Pl. Hartw. 306. 1848.—Herbaceous from a perpendicular root, the spreading stems 5–15 cm. long, dotted with small, flat, brown glands, soft-villous, as are the leaves: leaflets 9–13, oblong, cuneate-oblong, obovate, or obcordate, usually retuse, 3–8 mm. long, dotted with a row of small marginal glands: flowers numerous in oblong spikes, 1–6 cm. long; calyx 6 mm. long, its teeth filiform from a triangular base, equaling or exceeding the glandular-dotted tube; corolla rose-tinted, not exceeding the calyx teeth; banner 2 mm., wings and keel 3 mm. long, the latter mostly with a small gland at base: pod obovate, hirsute, and glandless, 3 mm. long: seed brown, reniform, 2 mm. long.—Fig. 1.

Probably a short-lived perennial, sometimes flowering the first year. The flowers are on very short pedicels, bracteolate at base by a pair of swollen pointed glands, which persist on the rachis after the fall of the fruiting calyces.

TYPE.—*"In vicinibus Monterey";* certainly an error. According to WATSON, in the *Botany of California,* it was first collected by COULTER, probably in southern Arizona.

DISTRIBUTION.—A common species in sandy and gravelly soils throughout the Lower Sonoran of the Colorado and Mojave deserts; thence northeast to southern Nevada (GOODDING 2237), southeast through Arizona (THORNBER)

and New Mexico to Coahuila, Mexico (PURPUS 115), south to Guaymas (PALMER) and Lower California (BRANDEGEE).

SPECIMENS EXAMINED.—*Mojave Desert:* Inyo Mountains, S. W. AUSTIN 441; Panamint Cañon, May 14, 1906, HALL 6999; Bagdad, May 2, 1892, BRANDEGEE; Camp Cady, May 1882, PARISH 83; Sheephole Mountains, May 1895, HALL 6097. *Colorado Desert:* Palm Springs, April 10, 1880, PARISH 88, and April 10, 1893, DAVIDSON; Coachella, April 1905, GREATA 415, and HALL 5792; Mecca, April 1904, Mrs. M. MCKIBBEN; McCoy Wash, April 1905, HALL 5932; Borregos Spring, April 28, 1894, BRANDEGEE; Hodges Mountain, April 1905, HALL 5975; Salton, April 12, 1892, DAVY 8049; Chuckawalla Bench, December 3, 1904, E. E. SCHELLENGER 99; Palo Verde, April 1905, HALL 5956; Calexico, March 29, 1902, DAVY 8005; Dixyland, October 18, 1912, PARISH 8312.

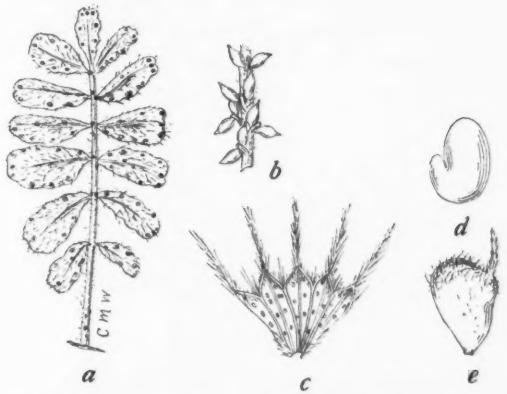

FIG. 1.—*Parosela mollis: a*, leaf; *b*, part of rachis, showing the glandular bracteoles; *c*, calyx, from the inside, to show the glands, which are concealed on the exterior by the dense hairs; *d*, seed; *e*, mature pod; *a*, *c*, *d*, *e*, ×3.

2. PAROSELA PARRYI (T. & G.) Heller, Cat. N. Am. Pl. ed. 2. 6. 1900.—*Dalea Parryi* T. & G., Proc. Am. Acad. **7**:397. 1868.—Perennial, the slender woody stems ascending, 1–6 dm. tall, finely and closely puberulent, or glabrate, dotted with small flat, dark glands: leaflets 3–21, oblong to obovate, obtuse or retuse, 1–3 mm. long, sparsely puberulent or glabrate, obscurely glandular-dotted or glandless: flowers numerous in pedunculate spikes 5–8 cm. long; calyx 3 mm. long, nervose, silky-canescent, obscuring the rows of small glands in the intervals, or glabrate; upper teeth triangular

or ovate-acute, the lowest narrower and longer, about half the length of the tube; corolla minutely glandular-dotted, twice exceeding the calyx; banner broadly orbicular, 1.5–2 mm. high and as

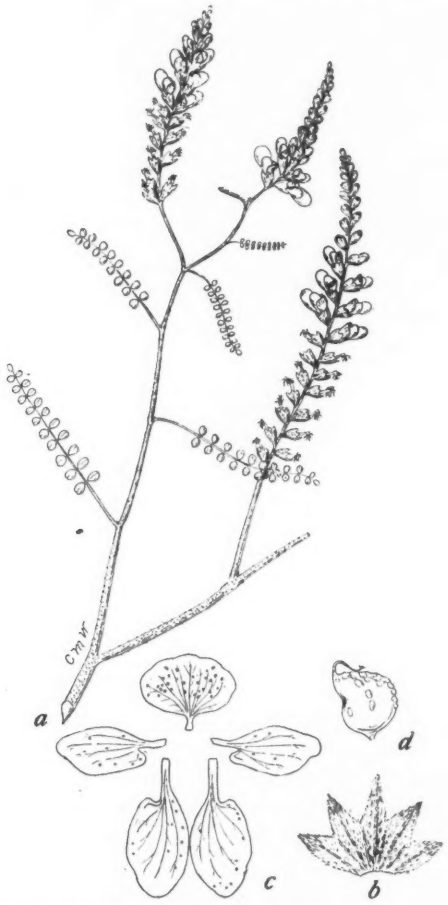

FIG. 2.—*Parosela Parryi: a*, branch, natural size; *b*, calyx; *c*, petals; *d*, pod; all ×2.5.

wide or wider; wings 2 mm. and keel 4 mm. long, the lower half of the banner and the upper half of the keel blue, the remainder white: pod gibbous, glandular, 2 mm. long: seed reniform.—Fig. 2.

TYPE.—"Gravelly hills near Ft. Mohave, Dr. J. G. COOPER, and lower down on the Colorado, near the mouth of the Williams River, Dr. C. C. PARRY."

DISTRIBUTION.—Lower Sonoran of the Colorado Desert, thence to Arizona (PURPUS 8507) and adjacent Mexico (PALMER) and Lower California (BRANDEGEE).

SPECIMENS EXAMINED.—Thermal, May 1903, DAVIDSON; Mammoth Tank, March 17, 1882, PARISH 1180; Cañon Springs Wash, February 25, 1904, SCHELLENGER 63; Cane Spring, April 1905, HALL 5846; Chuckawalla Bench, December 1906, SCHELLENGER 95; Palo Verde, April 1905, HALL 5975; Virginia Dale, May 1905, HALL 6041.

Parosela Orcuttii, comb. nov.—*Dalea Orcuttii* Wats., Proc. Am. Acad. **20**:359. 1885, a species of adjacent Lower California, may be looked for near the boundary. It resembles *P. Parryi*, but the stems are not glandular-dotted, and the corolla is but little longer than the calyx teeth.

†† *Flowers in condensed spikes which are sessile at the ends of branchlets, the rachises deciduous, leaving the branchlets as a subspinose armament; calyx teeth similar, or nearly so. Shrubs, even the old wood glandular*

3. PAROSELA POLYADENIA (Torr.) Heller, Cat. N. Am. Pl. ed. 2. 6. 1900.—*Dalea polyadenia* Torr. ex Wats., Geol. Expl. 40th Par. **5**:64. *pl. 9.* 1871.—Shrub 5–10 dm. tall, sparsely spinose, the short and stout divaricate branches canescent with a dense pannose tomentum, and copiously dotted with large yellow or red guttate glands: leaves on short petioles, 1–2 cm. long; leaflets 5–11, ovate, 1–5 mm. long, tomentose and sparsely glandular; flowers numerous; spikes globose or oblong; calyx villous and glandular-dotted, 3.5–4 mm. long; teeth subulate from a broad base, nearly as long as the tube; petals rose-pink or purple, each usually with a small gland at the apex, rarely somewhat bearded, 3.5 mm. long, little exceeding the calyx teeth: pod 3–5 mm. long, pubescent above.

TYPE.—"Borders of the Truckee Desert, Nevada."

DISTRIBUTION.—Inyo County, thence into Nevada (Wadsworth, KENNEDY, Candelaria, SHOCKLEY 275).

SPECIMENS EXAMINED.—Owens Valley, PURPUS 1960.

PAROSELA POLYADENIA (Torr.) var. **subnuda,** comb. nov.—*Dalea polyadenia* Torr. var. *subnuda* Wats., Bot. Cal. **2**:441. 1880.—Glabrous or nearly so; calyx glabrous and glandular-dotted, the teeth villous-ciliate.

TYPE.—"Owens Valley (Dr. W. MATTHEWS); Southern Utah (W. JOHNSON)."

SPECIMENS EXAMINED.—Mono County, July 1888, Mrs. J. H. HARCOURT; Owens Valley, S. W. AUSTIN 171; Owenyo, June 1911, DAVIDSON.

4. PAROSELA EMORYI (Gray) Heller, Cat. N. Am. Pl. ed. 2. 6. 1900.—*Dalea Emoryi* Gray, Mem. Am. Acad. II. **5**:315. 1855; Torr. Pac. R.R. Rep. **5**:360. *pl. 2.*—Shrub 1–2 m. high, destitute of true spines, the numerous slender intricate branches hoary pubescent and sprinkled with small brown or red prickle-shaped glands: leaves 1–9 cm. long, pubescent and sparsely glandular; leaflets 3–13, the terminal one usually narrower and longer than the lateral, rarely a few of the uppermost simple: flowers 10–20; spike globose, 1–2 cm. in diameter; calyx silky villous, 6 mm. long, colored orange by the abundant minute subulate glands; teeth linear, as long as the tube; corolla bright purple, little exceeding the calyx teeth; petals nearly equal, about 4 mm. long: pod 3 mm. long, dotted with red glands.

TYPE.—"On the desert tablelands of the Gila, 1852."

DISTRIBUTION.—Lower Sonoran of the Colorado Desert, thence into adjacent Arizona, and throughout Lower California to La Paz (BRANDEGEE).

SPECIMENS EXAMINED.—Palm Springs, April 1882, April 18, 1896, April 23, 1907, PARISH 93, 412, 6106; April 10, 1893, DAVIDSON; 1902, F. GILMAN; May 1894, L. D. COPELAND; April 20, 1906, GRANT; May 22, 1911, O. F. SELLIG; July 25–August 14, SCHELLENGER; Indio, May 8, 1903, JONES; McCoy Wash, April 1905, HALL 5946; Borregos Spring, April 19, 1906, JONES; abundant toward the foothills, Mecca, June 28, 1912, PARISH 8133; Old Beach, near Holtville, both above and below sea-level, June 30, 1912, PARISH 8088.

** *Ovules 2, collateral; pod glandular-dotted, exserted; the dead rachises persisting as a spinelike armament. Shrubs, the mature wood glabrous and glandless*

† *Flowers sessile, or nearly so, in open spikes; calyx teeth dissimilar, the lower one narrower and mostly longer than the upper pair; leaves pinnate, or a few of the uppermost simple*

5. **Parosela neglecta,** n. sp.—*Dalea arborescens* Parish, Zoe **4**:341. 1894, non TORREY.—Frutex subspinescens, 1–1.5 m. altus, caulibus junioribus tomentosis, cum glandulis parvis subulatis instructis; foliolis 3–7 oblongis vel obovatis sub tomento canescente obscure glandulosis; spicis 7–15-floris; calyce villoso minute glandulosoque, 8–9 mm. longo, tubo valde nervoso dentes aequante,

dentibus superioribus duobus acuminatis vel lanceolatis; corolla caerulea, vexillo oblongo-cordato, 8 mm. longo, 4 mm. lato; alis carinaque 6–7 mm. longis; ovario glanduloso-punctato.—Fig. 3.

FIG. 3.—*Parosela neglecta: a*, flowering branch, natural size; *b*, *c*, *d*, leaves; *e*, calyx from within; *f*, calyx exterior; *g*, petals; all ×2.5.

In recent years this plant has been taken for *Dalea arborescens* Torr., and in some respects it agrees with the character of that species given in *Plantae Thurberianae*, but is excluded by the phrases "*floribus in spicam densam brevem*

congestis . . . . spikes ovate or oblong," with which it does not in the least accord.

DISTRIBUTION.—So far as known, local in the neighborhood of Barstow, in the Mojave Desert.

Fishpond Station (Daggett), May 1882, PARISH 644, type; Barstow, May 14, 1897, F. W. HUBBY 141; May 3, 1906, HALL and CHANDLER 6831; May 18, 1905, HALL 6160.

6. PAROSELA JOHNSONII Vail, Bull. Torr. Bot. Club **24**:17. 1897.—*Dalea Johnsonii* Wats., Geol. Expl. 40th Par. **5**:64, 1871.—Shrub 1–1.5 m. high, the slender branches scantily appressed-pubescent or glabrate, glandless or nearly so: leaflets 5–7, linear to narrowly oblong, thinly pubescent and obscurely glandular, narrowed to the rachis: flowers short-pedicellate; calyx sparsely pubescent and nearly glandless, obscurely nerved, 5–5.5 mm. long, the teeth less than half the length of the tube, the upper pair triangular-acute; corolla deep purple; petals about 6 mm. long.

TYPE.—"Near St. George, on the Virgin River, Utah."

DISTRIBUTION.—From southern Utah to the borders of Arizona (Grand Cañon, WILSON) and the eastern border of the Colorado Desert.

SPECIMENS EXAMINED.—Eastern edge of the Colorado Desert, SCHELLENGER; Kane Spring, Ord Mountain, May 1, 1906, HALL and CHANDLER 6826; Cottonwood Mountains, May 11, 1905, HALL 6024.

PAROSELA JOHNSONII (Wats.) Vail var. **Saundersii,** comb. nov. —*P. Saundersii* Abrams, Bull. N.Y. Bot. Gard. **6**:396. 1910; *P. Wheeleri* Heller, Muhlenb. **2**:216. 1906; *Dalea Saundersii* Parish, Bull. S. Cal. Acad. **2**:83. *pl. 2.* 1903.—Leaflets 5–9, lanceolate, sessile, or on short petiolules, glabrate; stems sparsely prickle-glandular.

Victorville, May 12–14, 1903, C. F. SAUNDERS, and May 1905, HALL 6197; Big Pine, Inyo County, May 29, 1906, HALL and CHANDLER 7222; Owens Valley, May 11, 1906, HELLER 8236.

PAROSELA JOHNSONII (Wats.) Vail var. **pubescens,** n. var.—Calycis dentibus majoribus tenuioribusque; legumine pubescente simul glanduloso.

Lee's Ferry, Arizona, June 13, 1890, JONES 3076.

PAROSELA JOHNSONII (Wats.) Vail var. **minutifolia,** n. var.—Foliolis 5–7, oblongis, 2–4 mm. longis.

Mouth Panamint Cañon, May 11, 1906, HALL and CHANDLER 7002; Providence Mountains, May 24, 1905, BRANDEGEE.

7. PAROSELA CALIFORNICA Vail, Bull. Torr. Bot. Club **24**:17. 1897.—*Dalea californica* Wats., Proc. Am. Acad. **11**:132. 1876.—Shrub 1–2 m. high, hoary with a fine upwardly appressed pubescence, and sparsely beset with prickle-shaped glands: leaves 2–3 cm. long; leaflets 3–7, the terminal often longer than the others, rarely a few simple, the edges thickened, hoary-pubescent, concealing the small glands, decurrent on the rachis or confluent; calyx 5 mm. long, nerved, minutely glandular-dotted, thinly pubescent, in fruit glabrate; teeth shorter than the tube, the upper pair ovate-acute; corolla bright purple; petals 8–8.5 mm. long; pod 8 mm. long; seed castaneous, ovate.—Fig. 4.

Extreme forms, such as Jones's Palm Spring specimen (Hb. U. Cal. 12852), having leaves 3-foliolulate to simple, connect this species too closely with *Dalea Fremontii* Wats., and it is not impossible that it must be reduced to a variety thereof.

TYPE.—"Known as yet only from scanty specimens recently collected by Dr. PARRY in the San Bernardino Mountains, California." According to statements made to the writer by Dr. PARRY, the type was collected east of Banning, on the borders of the Colorado Desert.

DISTRIBUTION.—Western borders of the Colorado Desert, at 150–600 m. altitude, and in the eastern part of the San Jacinto Valley, in the cismontane area.

SPECIMENS EXAMINED.—Near Banning, 1882, PARISH 644, and May 1892, DAVIDSON; Palm Springs, April 1896, PARISH 4111, May 10, 1903, JONES, 1904, R. J. SMITH 308, May 21, 1911, O. F. SELLIG, and May 1902, HALL 1832; Cottonwood Mountains, May 1905, HALL 6025; between Palm Springs and Whitewater, July 25–August 14, E. E. SCHELLENGER, intermediate between the species and the following variety.

PAROSELA CALIFORNICA (Wats.) Vail var. **simplifolia,** n. var.—Glaberrima, ramis eglandulosis: foliis simplicis, rariusve pinnato-3-foliolatis; calycis majoribus.

Western part of the Colorado Desert, 1904, M. F. GILMAN 51 (in Herb. Univ. Cal.).

†† *Flowers pedicellate, in simple racemes; leaves all simple*

8. PAROSELA SCHOTTII (Torr.) Heller, Cat. N. Am. Pl. ed. 2. 6. 1900.—*Dalea Schottii* Torr. Bot. Mex. Bound. 53. 1859.—Compact spinose shrub 1–3 m. high, with numerous slender, green and glabrate, glandless branches: leaves linear, 3–25 mm. long, puberulent, but soon glabrate, bearing near the thickened margins a

few small, dark, impressed glands: racemes 4–8 cm. long, 6–20-flowered; pedicels 1–1.5 mm. long; calyx 5 mm. long, sparsely hirsute, glabrate in fruit, nerved, and with a row of small red glands,

FIG. 4.—*Parosela californica: a*, flowering branch, natural size; *b*, *c*, *d*, leaves; *e*, calyx and stamens; *f*, calyx laid open; *g*, petals; all ×2.5.

very obscure in flower, but distinct in fruit; teeth ciliate, the upper pair a little wider and more obtuse; corolla deep purple; banner 8 mm., wings 10 mm. and keel 8–10 mm. long: pod 1 cm. long, its glands, red: seed castaneous, obovate, 7–8 mm. long.—Fig. 5.

FIG. 5.—*Parosela Schottii: a*, flowering branch, natural size; *b*, calyx; *c*, petals; *a*, pod and fruiting calyx; all ×3.

Some of the lowest flowers are often in the axils of leaves. The fragrance of the abundant bloom is sometimes diffused for miles on the quiet desert air.

TYPE.—"Diluvial banks of the Colorado, February, SCHOTT."

DISTRIBUTION.—An abundant species of the Colorado Desert, extending into adjacent Arizona and into Lower California (BRANDEGEE).

SPECIMENS EXAMINED.—Palm Springs, April 10, 1880, April 1896, PARISH 83, 4113, April 1904, L. D. COPELAND 4, October 15, 1904, SCHELLENGER 3, 1902, M. F. GILMAN 21, April 1905, HALL 5738, and May 21, 1911, O. F. SELLIG; Coachella, April 1905, GREATA and HALL 5781; Chuckawalla Mountains, April 1905, HALL 5973; Indio, April 1905, HALL 5990.

PAROSELA SCHOTTII (Torr.) Heller var. **puberula,** n. var.—Rami juvenes foliaque canescente puberuli; calyce parum vel dense pubescente.

Colorado Desert, April 1905, BRANDEGEE; Borregos Spring, April 29, 1904, BRANDEGEE; Cajon de Santa Maria, Lower California, May 10, 1889, BRANDEGEE.

*** *Ovules 4 (–6); calyx teeth similar, pod glandular-dotted, exserted; flowers spicately scattered on stout spine-tipped branchlets* (*Asagraea* Baillon, Adansonia **9**:232. 1870)

9. PAROSELA SPINOSA Heller, Cat. N. Am. Pl. ed. 2. 7. 1900.—*Dalea spinosa* Gray, Mem. Am. Acad. **5**:315. 1855; Torrey, Pac. R.R. Rep. **7**[3]:9. *pl. 3.* 1856; *Asagraea spinosa* Baillon, Adansonia **9**:233. 1870.—Intricately branched tree 4–7 m. high, the numerous spinescent branchlets hoary with a fine close pubescence, and sparsely dotted with small flat glands; leaves very few and promptly deciduous, narrowly oblong, the margins thickened, 5 mm. long: flowers on pedicels 1 mm. long; calyx 5 mm. long, strongly nerved, encircled above with a ring of large, reddish, guttate glands; teeth 2 mm. long, ovate; corolla dark blue; banner 6 mm. long and as broad; keel and wings 8 mm. long; anthers with an oblong red gland at base.

The flowers do not extend to the sharp horny spine of the branchlets, not all of which are floriferous, and which cannot be regarded as the peduncles of a true spicate inflorescence. *Parosela Kingii* (Wats.) has solitary flowers borne on like spinescent branchlets, and *Holocantha Emoryi* Gray has an analogous inflorescence.

TYPE.—"Arroyos on the Gila; and on the California Desert west of the Colorado."

DISTRIBUTION.—At low altitudes in the Colorado Desert, thence to adjacent Arizona, Sonora, and Lower California.

SPECIMENS EXAMINED.—Colorado Desert, 1894, A. W. ANTHONY; Whitewater, May 1904, R. G. SMITH; Palm Springs, June 1895, DAVIDSON, in full flower, and April 1907, PARISH; Chuckawalla Bench, June 25, August 14, 1903, SCHELLENGER 2, 3; Indio, June 1880, PARISH 22; toward the foothills near Mecca, and in Red Cañon, abundant and in full bloom, June 28, 1912, PARISH 8108.

SPECIES INCERTA

DALEA ARBORESCENS Torr. ex Gray, Mem. Am. Acad. II. 5:316. 1885.—"Much branched, almost glandless, subspinescent; the adult branches glabrate, the younger, together with the leaves and the calyces, canescent-tomentose: leaflets 5, approximate, obovate: flowers congested in a short dense spike; bracts small, subulate; the acuminate teeth of the calyx as long as the campanulate tube, the two upper oblong-triangular, the others narrowly lanceolate: petals (purple?) about equal.

"'A small tree!' Glands scarcely any, a few minute tubercular ones occasionally found on the branchlets when denuded of their dense woolly covering. Leaves petioled, the leaflets only 2–3 lines long. Flowers 5–6 lines long; the calyx large in proportion, the tube obscurely striate. Vexillum obcordate."

The above is the original character, the first paragraph translated. The type is said to be from the "Mountains of San Fernando, a southern branch of the Sierra Nevada, California; April, FREMONT." This region is now well known, but no species of *Parosela* has been collected there; certainly it could hardly have escaped notice if a tree. The type specimen is a mere fragment, from which little can be learned. The condensed inflorescence indicates that it should be placed near *Parosela polyadenia* and *P. Emoryi*, where it was located by WATSON in the *Botany of California*.

SAN BERNARDINO, CAL.

THE EFFECT OF SOME PUGET SOUND BOG WATERS ON THE ROOT HAIRS OF TRADESCANTIA

GEORGE B. RIGG

The theory advanced in this paper is that plants other than bog xerophytes are excluded from peat bogs because of their inability to produce normal root hairs in the toxic habitat of bogs, their absorptive surface being thus so decreased that they cannot get water enough to enable them to live. The writer has also confirmed with Puget Sound bog waters certain results obtained by other workers with bog waters from the Middle West and extreme East.

Description of bogs

The xerophilous character of the flora of peat bogs is well known. In the Puget Sound region the plants most characteristic of undrained bogs are *Ledum groenlandicum*, *Kalmia glauca*, *Oxycoccus oxycoccus intermedius*, *Sphagnum*, and *Drosera rotundifolia*. The first four plants named are found in every undrained bog that the author has visited in the region, while the last one has been found absent from a few. Other plants sometimes found in bogs of the region are *Pinus monticola*, *Betula glandulosa*, *Salix myrtilloides*, *Myrica Gale*, *Eriophorum russeolum*, and *Juncus oregana*. *Tsuga heterophylla* and *Pinus contorta* are found on the drier hummocks in bogs (**8**), *Ledum columbianum* and *Myrica californica* are reported to be found in the beach bogs along the Pacific Ocean instead of *L. groenlandicum* and *M. Gale* (**8**).

Peat bogs are common in the Puget Sound region. The studies reported in this paper are based on six bogs. One of these is situated within the city of Seattle at the corner of E. 55th St. and 6th Ave. N.E. During the last two years the forest surrounding this bog has been cleared away and the streets along its edges have been filled with dirt from the neighboring hills. It has not been drained however and its flora is still just as typical as it was before the surrounding forest was removed, except that *Drosera*

rotundifolia has disappeared, which was fairly common in this bog in 1908. It is a small bog, about 100 by 200 m. in extent. For convenience it will be referred to as the Seattle bog.

The largest bog studied is situated about 1760 m. east of Henry Station on the Seattle-Everett interurban railway. The undrained portion of this bog is perhaps 16 hectares in area, and its flora is typical, including *Drosera*. In addition to the usual bog plants, *Pinus monticola* is common, and *Trientalis arctica* is found along the border. The natural contour of the region is such that there is some drainage from the northern end of this bog into a small creek, and that portion of it lacks *Drosera* and *Pinus* and has *Lysichiton kamtschatcense*. This bog will be referred to as the Henry bog.

The bog situated about 5280 m. southeast of Fauntleroy Park in Seattle is a little smaller than the Henry bog. Its area is estimated at about 10 hectares. *Drosera* is abundant in it, and *Trientalis arctica* is common along its margin. This bog will be designated as the Fauntleroy bog.

The bog situated at Echo Lake station on the Seattle-Everett interurban railway is slightly smaller even than the Seattle bog. It is on the margin of Echo Lake and its edge forms the bank of the lake for a short distance. The bog flora is typical right up to the open water of the lake; *Drosera* is abundant, and *Comarum palustre* is found on the border of the lake immediately adjacent to the bog. This bog will be called the Echo Lake bog. It is about 8800 m. distant from the Henry bog.

The bog that will be referred to as the Green Lake bog is situated just north of the city limits of Seattle. It is a little over 1760 m. north of Green Lake, which is entirely within the city limits. There is now remaining only about 0.4 hectare of this bog; formerly it was about 12 hectares in extent, but nearly all of it has been stripped of its original vegetation and drained. A good deal of it has been divided into small garden tracts and some of these are now under cultivation. The uncleared portion is drained by roadside ditches on two sides; water flows freely from the bog into one of these ditches during the rainy season and there is considerable seepage into the other one. This bog still has the typical bog

plants of the region, including *Drosera*. In addition to these, however, it contains the following plants not usually found in bogs: *Pseudotsuga taxifolia*, *Picea sitchensis*, *Thuja plicata*, *Tsuga heterophylla*, *Alnus oregona*, *Comarum palustre*, *Lysichiton kamtschatcense*, a *Carex*, and a small orchid. It will be noted that some of them (e.g., *Lysichiton kamtschatcense*) are found on the borders of other bogs, and that one of them (*Tsuga heterophylla*) is found on the dry hummocks in other bogs. Apparently the partial drainage here has allowed the entrance of plants not found in typical bogs, but has not driven out the typical bog plants.

The last of the six bogs is situated on Mount Constitution at an elevation of about 666 m.; the mountain itself attains a height of 880 m. It is situated on Orcas Island, one of the San Juan group, which lies between the strait of Juan de Fuca and the strait of Georgia. Several peat bogs situated on this mountain have been drained and thus converted into meadows, which have been used for both hay and pasturage. Drainage and clearing seem to have completely destroyed the bog flora and substituted a flora not at all characteristic of peat bogs.

The bog water for use in the experiments has been obtained in all cases as follows. Care was first taken to select a spot that was centrally located in the bog and had a typical bog flora. The mass of vegetation and fibrous peat was cut away with a strong knife from an area about one foot square. Then the soft peat was scooped out below this until a cavity was formed that would soon fill with water. The water was dipped up in a wide-mouth glass bottle and poured into glass containers. The depth to which this had to be scooped out varies with the season; in winter about 35 cm. sufficed in most of the bogs; in late summer it was necessary to dig 90 cm. In the case of the water obtained from the Henry bog on October 10, 1911, it was found that it would not accumulate in half an hour by digging even 90 cm. deep, and a glass jar was filled with very wet peat and the water was squeezed through cheesecloth in the laboratory.

The tap water used was that supplied to the laboratories from a wooden supply tank on the University campus. It is pumped into this tank from Lake Washington.

The expression "normal root hairs" used in this paper with reference to *Tradescantia* (the species common in greenhouses, and known as wandering Jew) means such root hairs as grow on the roots of cuttings in tap water. These hairs cover the entire surface of the root even when it reaches a length of 70 mm. or more. They are almost uniformly distributed, 4 mm. or more in length, mostly straight, and appear to the naked eye like somewhat silky fibers. Over 200 specimens of this plant grown in tap water have been examined and there has been found practically no variation from this description.

Investigation

In October 1909 experiments were begun on the germination of wheat, corn, beans, and peas in moist peat and between sheets of moistened blotting paper. It was found that these seeds germinated just as readily when the moisture was furnished by bog water as when it was furnished by tap water.

In the fall of 1910 *Ledum groenlandicum* and *Kalmia glauca* were propagated by cuttings in both bog water and tap water in the laboratory. Young roots from both of these species from both kinds of water were examined and found to be entirely devoid of root hairs. Some of the roots examined were produced on old roots formed before the plants were removed from the bog and some were produced on stems.

TRANSEAU (10) found root hairs absent in *Oxycoccus macrocarpus*. He also found that the roots of *Larix laricina* were "composed of mycorhiza," and that their cortical tissues were early destroyed by a fungus. When he grew these plants in a well-aerated culture solution "normal roots with root hairs were produced."

COVILLE (1) found *Vaccinium corymbosum* to be devoid of root hairs. He found also that the walls of the epidermal cells of the roots were 1.3–2.5 μ thick, this being four to six times as thick as the walls of epidermal cells of wheat roots. He computes that a given section of wheat root presents about ten times as much absorptive surface as a section of blueberry (*Vaccinium corymbosum*) root of the same area.

In the fall of 1909 and again in 1910–1911 experiments were conducted on the effect of bog water on the production of root hairs on cuttings of *Tradescantia*. The experiments reported in the following table were carried out in 1910–1911 in 150 cc. glass bottles with extra wide mouths.

TABLE I

THE PRODUCTION OF ROOT HAIRS ON TRADESCANTIA IN BOG WATER

	No. of plants used	Root hairs			
		Normal	Slightly stunted	Much stunted	None
Seattle bog	16	..	3	9	4
Henry bog	10	..	1	9	..
Fauntleroy bog	10	..	3	6	1
Echo Lake bog	5	..	..	2	3
Green Lake bog	10	10	..	..	..
Mount Constitution bog	3	..	..	3	..

It will be noted that the Green Lake bog is the only one whose water allowed the production of root hairs that were normal as to length and abundance. It seems evident that this lack of toxic effect is a result of drainage. Of the 44 plants grown in water from undrained bogs, 8 plants (18 per cent) produced no root hairs, while 29 plants (66 per cent) produced root hairs that were much stunted, and 7 plants (16 per cent) produced root hairs that were slightly stunted.

The above table is based on the roots produced within the first 14 days; these roots were invariably shorter than those produced in tap water within the same time. The new roots that started after that time approximated the length of the roots of plants grown in tap water and produced root hairs that were longer and much more abundant, in many cases approximating normal. The tops of the bottles in which these experiments were carried on were not closed, the surface of the water being exposed freely to the air. DACHNOWSKI (**2**) found that aeration reduced the toxic effect of bog water from Cranberry Island (Ohio).

In addition to the tests made on bog water and tap water the following tests have been made on other waters of the region: Echo

Lake (5 plants), bog spring on Mount Constitution (3 plants), Mud Lake (10 plants), well water at Friday Harbor, Washington (25 plants). In every one of these cases the root hairs were normal. The water from Echo Lake was dipped up by the writer while standing on the edge of the Echo Lake bog; it was obtained at a distance of 15 feet from where the water from the Echo Lake bog was obtained. The bog spring on Mount Constitution referred to emerges from the side of the mountain and its water seeps into the swamp which gradually merges into the bog. It has the coffee color characteristic of bog water, but not to so marked a degree as the water obtained from underneath typical bog vegetation.

Mud Lake is so close to Lake Washington that the two are connected during the winter season. It is a circular lake about 880 m. in diameter. There is some bog vegetation near its southern end, and the situation of this vegetation appears to be in a general way similar to that of Buckeye Lake bog in Ohio described by DACHNOWSKI (**5**). The water used was obtained from the edge of the lake at a distance of 90 m. or more from the bog vegetation. The well water used at Friday Harbor was obtained from a surface well near the Puget Sound Marine Station. This is called "tap water" in table I, since the effect of the two has been found to be identical.

Plants were also grown in several solutions which it was expected would prove toxic. The following list of substances was found to produce stunting of the root hairs of *Tradescantia* of an amount and kind comparable with the effect of undiluted bog water: sea water diluted with three times its volume of tap water; carbolic acid, 0.001 per cent; formalin, 0.001 per cent; gelatin, 0.001–0.002 per cent; tea; coffee. In undiluted sea water no roots developed. Stronger solutions of carbolic acid and of formalin entirely inhibited the development of roots.

It is of course possible that formalin might develop from the slow decay of woody materials in the bog in the absence of oxygen, but I have not found any evidence of its presence in bog water. We might reasonably expect, also, that tannin would be found in bogs, but we have no direct evidence that it is a factor in limiting bog floras.

The effect of dilution with tap water was tried with the results shown in the following table:

TABLE II

THE EFFECT UPON ROOT HAIRS OF TRADESCANTIA OF BOG WATER DILUTED WITH TAP WATER

Bog	No. of exp.	Amount of dilution	Root hairs			
			Normal	Slightly stunted	Much stunted	None
Seattle bog.....	1	$\frac{3}{4}$	1	..	..	..
Seattle bog.....	11	$\frac{1}{2}$	11	..	..	..
Henry bog.....	10	$\frac{1}{2}$	7	3	..	..
Fauntleroy bog	10	$\frac{1}{2}$	10	..	..	..
Fauntleroy bog	5	$\frac{1}{4}$	5	..	..	..
Fauntleroy bog	10	$\frac{1}{10}$	..	5	4	1

From this it appears that the toxin is present in such small amounts that slight dilution greatly decreases the toxic effect. This is in line with the results obtained by LIVINGSTON (**7**) on *Stigeoclonium*.

Three samples of water from the Henry bog were boiled until each was reduced to one-eighth of its original volume. *Tradescantia* cuttings were placed in them. Few roots started, no root hairs were formed on them, and the plants soon died.

The effect of filtering bog water from the Henry bog through filter paper was tried. The water was collected on October 10, 1911, and three plants were grown in it. They all produced normal root hairs.

A preliminary investigation was made as to the presence and activities of bacteria in the Seattle bog and the Henry bog. Briefly the results may be stated as follows.

1. Beans, peas, and corn decay just as readily in bog water as in tap water.

2. Fresh beef decays a little more slowly in bog water collected in a sterilized jar and kept sealed than it does in tap water under the same conditions.

3. The amount of difference in the rate of decay of pieces of fresh beef buried in bogs and of other pieces buried in swamps is very slight, it being a little more rapid in swamps.

4. Bacteria were found in every case in both bog water and peat collected under sterile conditions. Some of the specimens of peat were collected from as great a depth as 75 cm.

5. *Bacillus subtilis* and *Pseudomonas liquefaciens* were identified in cultures made from surface waters in the Seattle bog.

TRANSEAU (**9**) found bog waters to be teeming with bacteria. DACHNOWSKI (**4, 5**) has found bacteria abundant and has given his attention largely to their physiology. Apparently the position that bog waters are very strongly antiseptic is no longer tenable.

Discussion

Suggestions offered by three other investigators (LIVINGSTON, DACHNOWSKI, and COVILLE) bear on the theory stated at the beginning of this paper. In 1905 LIVINGSTON (**7**) concluded that there were chemical substances present at least in some bog waters that affected the alga that he used (*Stigeoclonium*) as did poisoned solutions, and that these substances are not related directly to the acidity of the water. He concludes that "the stimulating substances here demonstrated may play an important rôle in the inhibition from bogs of plants other than those of xerophilous habit." In 1909 DACHNOWSKI (**3**) stated his belief "that there are present in bog water and in bog soils injurious substances which are, at least in part, the cause of xerophily in plants and of decreased fertility in bog soils." In 1910 COVILLE (**1**) stated that "the swamp blueberry (*Vaccinium corymbosum*) grows in peaty soils which contain acid or other substances poisonous to plants. As a protection against the absorption of amounts of these poisons great enough to prove fatal, this plant, like many other bog and acid-soil plants, is devoid of root hairs and consequently has a restricted capacity for absorbing soil moisture." In 1911 DACHNOWSKI (**5**) words his theory a little differently and speaks of "the toxicity of the habitat and its consequent physiological aridity and selective operation on forms striving for occupancy." In the same paper DACHNOWSKI says that "the reduced absorptive capacity of the plants is not a consequence of the absence of root hairs or of a smaller absorbing surface."

It is thus seen that LIVINGSTON suggested that bog toxins

excluded certain plants from bogs, but did not express any opinion on root hairs, while COVILLE stated the theory that certain plants were devoid of root hairs as a protection against bog poisons, but does not give an opinion whether the bog habitat as it at present exists caused the loss of these root hairs. Neither does he express any opinion as to how mesophytic plants are kept out of bogs. It is to be borne in mind that COVILLE was working on a specific economic problem and evidently did not concern himself, in the paper quoted, with questions of pure science. DACHNOWSKI at first thought that toxins caused xerophily in bog plants and later that the toxicity caused bogs to exercise a selective operation, but does not suggest any injurious effect of bog toxins on root hairs as the cause of such selective operation.

TRANSEAU'S work (**10**) would seem to suggest that *Larix laricina* is adapted to the Michigan bogs because it can still live after the loss of its root hairs and even after the destruction of the cortical tissues of its roots. *Larix*, however, is not a genus that is universally characteristic of bogs as are such genera as *Ledum*, *Kalmia*, *Oxycoccus*, and *Vaccinium*. There seems to be room for doubt as to the cause of the death of the root hairs and of the cortical tissues of the roots of *Larix* in the Michigan bogs. It is possible that they may be killed by a toxin and attacked by a saprophytic fungus afterward. It is also possible that they may have been killed by a parasitic fungus.

Definite conclusions as to the relation of the toxicity of the bog habitat as a cause and the stunting of root hairs as a result cannot, of course, be drawn from the results obtained from the use of water from six bogs on a single species. Further work must be done with other bog waters and other plants to show how far these two things are related as cause and effect. The question of how bog plants came to be devoid of root hairs is quite a different question from that as to why mesophytic plants are now excluded from undrained bogs. DACHNOWSKI, who in 1909 (**3**) believed in the activity of bog toxins in causing xerophily in bog plants, states (**5**) in 1911 that "during the glacial period most species common to bogs skirted the ice sheet." Whether these plants were under bog conditions at this time or whether their distribution was related

to low temperatures only does not seem to be settled. Evidently extremely low temperatures must be reckoned with as one of the factors that determine the characteristics of these plants in past ages, and the same is true of bog plants growing in the extreme north in post-glacial times. We certainly are not justified in concluding that bog conditions as they exist today in temperate regions are the cause of xerophily in bog plants. There does seem to be ground for the belief that certain plants having hairless roots and other xerophilous structures are able to live in bogs, while other plants that normally have root hairs and possess in general a mesophytic or tropophytic structure are kept out of the bogs by these toxins.

It now seems well established that the inhibition from undrained bogs of plants other than xerophytes is not caused by acidity as such (H ions) (LIVINGSTON **7**), nor by low osmotic pressure (LIVINGSTON **6**), and that it cannot be correlated with low temperatures or strong drying winds (DACHNOWSKI **5**), or directly with lack of aeration. Although the toxic effect of bog waters does disappear with continued aeration (DACHNOWSKI **2**), it seems evident that the presence of air destroys the toxic substances that are present in bog water, and that the mere absence of air from water does not render it toxic. The fact that DACHNOWSKI (**5**) found that the toxic effect of bog water can be removed by filtering it through agricultural soils and that the toxic effect was then present in the soil used as a filter seems to settle the point. Whether the toxic effect of bog waters is due to one substance or to several we do not know. Nor do we know positively that it is always due to the same substance or mixture of substances. Undoubtedly the toxic substances are organic, and the problems of organic analysis involved are beyond us at present.

What the source of the toxin (or toxins) is we do not know definitely. There seem to be at least three possible sources: (*a*) excretion products coming into the substratum from plants growing in the bog, (*b*) products resulting from decay in the absence of oxygen, (*c*) excretion products of bacteria. Since it is probable that many other fungi are associated with the bacteria in bogs, it seems scarcely possible to distinguish sharply between (*a*) and (*c*).

Since *Sphagnum* is the one macroscopic plant always present in bogs, our attention would naturally be directed to that. Since the presence of *Sphagnum* and the lack of drainage are the two conditions necessary for the formation of bogs, it seems probable that in this combination is the place to seek for the production of the toxin. Bacteria, however, seem to be always present in bogs and their excretion products are to be taken into account.

DACHNOWSKI (**5**) finds reason for believing that bacteria are active agents in enabling peat bogs to admit certain plants and exclude others. As the same investigator (**4**) has suggested, the large number of chemical and biological agents present may react collectively with the results of decomposition. Since it has been found by DACHNOWSKI (**3**) that the presence of a considerable amount of a finely divided insoluble substance destroys the toxic effect of bog water, it seems possible that the absence from bogs of ordinary insoluble soil substances may be a factor in the production of toxicity in bogs.

DACHNOWSKI (**5**) has given recently a historical summary of the theories of the causes of the xerophilous character of bog plants. In this summary he says "LIVINGSTON suggests the presence of chemical substances not in direct relation to the acidity of the soil as acting on the vegetation. Another explanation, that of the toxicity of the habitat and its consequent physiological aridity and selective operation upon forms striving for occupancy, has been offered by the writer of this paper." In the paper above quoted LIVINGSTON says "the result of these tests is, briefly, that many bog waters act upon the plant [*Stigeoclonium*] like *poisoned* solutions." Again, he says "diluting the . . . . samples . . . . with distilled water or with a weak nutrient solution decreases the *toxic* effect." In his summary he says "the *stimulating* substances here demonstrated may play an important rôle in the inhibition from bogs of plants other than those of xerophilous habit."

It seems to the writer that the toxin theory of the cause of the exclusion from bogs of plants other than certain xerophytes originated with LIVINGSTON. The theory has been greatly extended and a wealth of experimental data given to support it by DACHNOWSKI,

and the present paper contributes the suggestion that the toxins act through their stunting effect on root hairs.

The bog problem was suggested to the writer by Professor THEODORE C. FRYE, of the University of Washington, and he has had the advantage of his criticism and advice as well as that of Dr. JOHN WEINZIRL, bacteriologist in the same department.

Summary

1. *Tradescantia* grown in bog water shows stunted root hairs.

2. *Tradescantia* grown in water from open lakes and springs immediately adjacent to bogs shows normal root hairs.

3. *Tradescantia* grown in water from drained or partly drained bogs shows almost normal root hairs.

4. The stunting of root hairs of *Tradescantia* by bog water is comparable with the stunting of them by exceedingly dilute solution of sea water, of formalin, of tannic acid, of gelatin, of coffee, and of tea.

5. The stunting effect of bog water on root hairs of *Tradescantia* disappears when it is diluted with an equal volume of tap water and in some cases when diluted with one-half its volume of tap water.

6. The stunting effect of bog water on root hairs of *Tradescantia* may be increased by boiling the water down to a fraction of its original volume.

7. Many typical bog plants have no root hairs.

8. There seems to be a toxin or toxins in bog water whose effect disappears with drainage of the bog.

9. Possibly this toxin inhibits mesophytes from bogs by reducing the amount of absorptive surface exposed by the root system.

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CURRENT LITERATURE

BOOK REVIEWS

The ecology of water plants

Long ago the ecological features of the hydrophytes were brought together in comprehensive fashion by SCHENCK in two admirable volumes. Recently Dr. HUGO GLÜCK, a young and enthusiastic investigator, has published three volumes along similar lines, and they are very rich in detailed information; indeed, they may be regarded as encyclopedic in nature. The first two volumes, dealing respectively with the European Alismaceae and the genus *Utricularia* (together with an account of turion formation), have been noticed in these pages.[1] The third and thus far the largest volume, which is now at hand, is very different in scope, treating as a whole the vegetation of fresh-water banks, that is, the marginal vegetation of streams and ponds.[2] This vegetation is essentially that of the belts which are subject to inundation, and embraces, therefore, practically all of the so-called amphibious plants. These plants, of course, are among the most interesting of all plants to ecologists because of their plasticity.

While most of the genera and many of the 124 species treated in this volume are of very widespread distribution, the author limits his studies to central and southern Europe. The work is based on almost numberless field trips to all parts of this vast region, and these observations have been backed up by numerous cultures. The author makes no pretense to completeness, suggesting, indeed, that these are among the most poorly known of all plants, and that they are well worthy of much more careful study; some color is given to this view by GLÜCK's discovery, in these floristically best known of all lands, of entirely new areas for four different species. The author recognizes two "zones" (thus ignoring the Brussels recommendation of 1910 that the term zone be employed henceforth only for the great climatic belts of the earth), one of the land margin with leaves mostly aerial, and one of the water margin with leaves mostly submersed. The subdivision of the "zones" into groups and subgroups is based not on habitat but on leaf form. For example, "zone 1" includes a group with linear leaves (as *Typha*, *Acorus*, *Iris*), a group with petioled entire leaves, a group with petioled divided leaves, etc. Under each group or subgroup the species are considered individually, and under each

[1] BOT. GAZ. **43**:67–69. 1907.

[2] GLÜCK, HUGO, Biologische und morphologische Untersuchungen über Wasser- und Sumpfgewächse. III. Die Uferflora. pp. xxxiv+644. *pls. 8. figs. 105.* Jena: Gustav Fischer. 1911.

species there is usually a further subdivision into such topics as land form, water form, submersed form, form with floating leaves, etc. The greater part of the work is taken up by the plants of the water margin ("zone 2"). These belong to two categories, so far as leaf form is concerned, those that are homoblastic or with but one leaf type, and those that are heteroblastic or with two leaf types; the heteroblastic water plants are with us well represented by such plants as *Sium* and *Proserpinaca*. Most of the plants of "zone 1" have greatly reduced water forms, many of which are generally unfamiliar, and some of which are known only from cultures; these forms rarely flower. One of the striking discoveries is a water form of a dodder (*Cuscuta alba*) which parasitizes *Isoetes* and water buttercups. Only a few of the species studied live in flowing water. The water forms seem related to low temperatures, and while the land form is essentially a summer form, it can sometimes be produced at other seasons by raising the temperature of the cultures. Most of the species have winter rest periods, but there are some species that vegetate continuously, even in countries with cold winters. Many Mediterranean species have periods of summer rest. These and many other topics are considered in the 40-page summary with which the volume concludes. The book should be in every botanical reference library, for it will serve as a compendium of general ecological information about the plants it treats. It is understood that Dr. GLÜCK is devoting his life to the study of water plants, and we may expect other volumes of this sort in the future.—HENRY C. COWLES.

Plant breeding in Sweden

The extensive series of experiments in plant breeding which have been conducted in Sweden, principally at the Svalöf station, beginning about 1886 and extending with ever increasing efficiency to the present time, are of great interest not only to agriculturists but also to scientists by reason of the problems of inheritance which they involve. Unfortunately very scanty reports of these operations have been available in any but the Swedish language, and these publications have been, and quite rightly so, most largely concerned with practical results that were of special interest to the farmers of Scandinavia. These circumstances will make the present report[3] the more useful, prepared as it is by one who has carefully investigated the methods employed and the results obtained at the Swedish stations, and addressed primarily to the scientific reader, but in language intelligible to the general public.

The report contains a brief historical sketch of the inception and development of plant breeding experimentation in Scandinavia, examples of the experiments with different agricultural plants, some of the results obtained, and a summary of the principles now recognized by the plant breeders at Svalöf and followed in their work. These principles are briefly: (1) the recognition

[3] NEWMAN, L. H., Plant breeding in Scandinavia. 8vo. pp. 193. *figs. 63*. Ottawa, Canada: Canadian Seed Growers' Association. 1912. $1.00.

that a progressive system of plant improvement cannot be a one-sided system, but must embrace all possible methods of reaching the desired end; (2) artificial hybridization provides an invaluable means of obtaining characters in superior combinations which do not occur in nature and this method is now largely used at Svalöf for this purpose; (3) the old system of "mass selection" can still be of value in special cases and has never been fully abandoned; (4) instead of basing the isolation of superior individuals (or lines) purely upon botanical or morphological characters, as was formerly done, the principle has become to select a large number of individuals without special regard to such characters, the valuation of these individuals (or lines) to rest upon tests conducted with the greatest care and extending over a series of years. This means the recognition of the importance of physiological as well as morphological unit characters, and the abandonment of reliance upon the use of correlation of characters as any important aid in estimating the practical value of an individual or line.

Detailed reports of some investigations, some sixty illustrations from drawings and photographs, and a comprehensive bibliography add to the value of the volume. Dr. TEDIN, specialist for barley at Svalöf, says of the book: "The exposition is pertinent and correct and thereby distinguishes itself from practically all accounts hitherto written in a foreign language."—GEO. D. FULLER.

The cotton plant

W. LAWRENCE BALLS, "cryptogamic botanist" on the staff of the Khedivial Agricultural Society, has published a volume on the cotton plant in Egypt.[4] It brings together information of the most varied character, the material being assembled as if to "take account of stock" preliminary to a fuller monograph. The four sections of the book treat of the history of cotton in Egypt, the individual plant, the race, and the economics of cotton, the second and third sections being of special interest to botanists.

In the account of "the individual plant" a brief outline of fertilization (including the conspicuous cytological features) and embryology is given (8 pp.), followed by an account of experimental work on "development and environment" (67 pp.). This physiological work includes such topics as germination conditions, temperature and growth, effect of sunshine, night temperatures, hypocotyl and root growth, transpiration (including its relation to stomata), tissue temperatures, photosynthesis, the growth curve, the flowering curve, etc. The cotton fiber of course is described in detail (8 pp.). In the account of "the race," the problems of fluctuation, commercial varieties, natural crossing, and heredity are presented (87 pp.), quite a number of graphs presenting to the eye the results of much experimental work.—J. M. C.

[4] BALLS, W. LAWRENCE, The cotton plant in Egypt; studies in physiology and genetics. pp. xvi+202. *figs.* 71. London: Macmillan & Co. 1912. 5 *s.*

Tree manuals

Among the many books upon various phases of tree study two recently to hand seem well suited to the use for which they were intended. The first[5] includes the native and cultivated woody plants of Minnesota, and in its semitechnical nature is intended to appeal to the educated layman quite as much as to the teachers and students of botany and forestry. This appeal is the more readily made through the numerous excellent illustrations, both from drawings and photographs.

In the second manual[6] the same classes are appealed to, and in addition to simplicity of text and abundance of excellent illustrations, the book has further to recommend it, its pocket-size, which allows it to be most conveniently carried into the forests themselves. The leather covered edition is in fact the best and most portable small tree manual that has yet appeared. —GEO. D. FULLER.

MINOR NOTICES

A sketch of Linnaeus.—Professor EDWARD LEE GREENE[7] has published an admirable sketch of LINNAEUS, which the publishers have presented in most attractive form. The personality of this great Swede should live beyond the circle of professional taxonomists, and the simple and fine style of this sketch makes the booklet a most effective one for the teachers of public schools and for reading circles. The nine sections deal with the following topics: lineage and childhood of LINNAEUS; school, college, and university years; journey to Lapland; journey to Germany and Holland; practices medicine in Stockholm; appointed professor at Upsala; influence of LINNAEUS upon botany; LINNAEUS as a zoologist (contributed by W. H. DALL); LINNAEUS as an evolutionist. These sections introduce LINNAEUS the man rather than as the father of taxonomy, and a most interesting and inspiring man he proves to be. We are told of his parentage and early education, of his struggles with adverse circumstances, and of the almost incredible patience, industry, zeal, and resolution with which he conquered and rose to high distinction. No one could introduce him more intelligently, sympathetically, and in better form than Professor GREENE.—J. M. C.

Indiana Academy of Science.—The Proceedings of the Indiana Academy of Science for 1911 (1912), a volume of 473 pages, contains the following botani-

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[5] CLEMENTS, F. E., ROSENDAHL, C. O., and BUTTERS, F. K., Minnesota trees and shrubs. Report of the Botanical Survey IX. 8vo. xxi+314 (illustrated). Minneapolis, Minn.: University of Minnesota. 1912.

[6] COLLINS, J. F., and PRESTON, H. W., Illustrated key to the wild and commonly cultivated trees of the northeastern United States and adjacent Canada. vii+184. *figs. 279.* New York: Henry Holt & Co. Cloth $1.35; leather $2.50.

[7] GREENE, EDWARD LEE, Carolus Linnaeus. pp. 91. Philadelphia: Christopher Sower Company. 1912.

cal papers: Some variations in plants, by F. M. ANDREWS; Report of the work in corn pollination (III), by M. L. FISHER; New and notable members of the Indiana flora, by E. J. GRIMES; A monograph of the common Indiana species of *Hypoxylon*, by CHARLES E. OWENS; The improvement of medicinal plants, by F. A. MILLER; Nutrients in green shoots of trees, by E. J. PETRY; The New York apple tree canker, by LEX R. HESLER; Value of fertilizing constituents of weeds of Indiana; analysis of ironweeds, by FRANK MATHERS and Miss GAIL M. STAPP; The prevalence and prevention of stinking smut in Indiana, by C. T. ORTON; Indiana fungi (II), by J. M. VAN HOOK; Diseases of ginseng caused by Sclerotinias, by GEO. A. OSNER; Additions to the flora of the Lower Wabash Valley (by Dr. J. SCHNECK), by CHARLES C. DEAM; Plants new or rare in Indiana, by CHARLES C. DEAM; The unattached aerial forms of plant rusts in North America, by A. G. JOHNSON.—J. M. C.

Sylloge Fungorum.[8]—Volumes XIX and XX of this extended work, bearing the subsidiary title *Index Iconum Fungorum*, contains a bibliographical index to illustrations of fungi, and includes references to works of many of the early as well as the more recent authors. References to illustrations are indicated briefly but clearly; synonyms are introduced frequently and serve as a ready and unmistakable means of cross reference. Volume XIX enumerates alphabetically the genera *Abrothallus* to *Lysurus* inclusive, and Volume XX continues with *Macowanites* to *Zythia*. The species under their respective genera and the bibliographical references thereto are likewise in alphabetical sequence, and the terminology is in accordance with the international rules of botanical nomenclature. The amount of detailed and painstaking labor involved in the achievement of such a task is enormous, but the final result in this case is a valuable work of reference, indispensable to the mycologist and helpful to the general student of botany.—J. M. GREENMAN.

NOTES FOR STUDENTS

Current taxonomic literature.—C. A. DARLING (Torreya **12**:155–164. 1912) has issued a "Key to the wild and cultivated trees in autumn." The key is intended for use in the field for the identification of trees occurring in eastern United States.—A. DAVIDSON (Bull. So. Cal. Acad. Sci. **11**:77. *pl. 1.* 1912) describes and illustrates a new species of *Frasera* (*F. puberulenta*) from California.—B. O. DODGE (Mycologia **4**:218–222. *pls. 62, 63.* 1912) describes and illustrates a new species of *Ascobolus* (*A. magnificus*) from artificial cultures

[8] SACCARDO, P. A., Sylloge Fungorum omnium hucusque cognitorum. Vols. XIX and XX. *Index Iconum Fungorum* enumerans eorundem figuras omnes hucusque editas ab auctoribus sive antiquis sive recentioribus. Ductu et consilio P. A. SACCARDO. Congessit J. B. TRAVERSO. Roy. 8vo. Vol. XIX, pp. xi+1158; Vol. XX, pp. 1310. Sumptibus P. A. SACCARDO. Typis Seminarii. Patavii, 23 March 1910, and 25 May 1911.

conducted in New York City.—J. R. DRUMMOND (Bot. Mag. *t. 8451.* 1912) describes and illustrates a new species of *Agave* (*A. disceptata*) supposed to be native of Central America.—A. A. HELLER (Muhlenbergia **8**:82–84. 1912) in continuation of studies on the genus *Lupinus* records a new species (*L. borealis*) from the Yukon region, Canada.—W. A. MURRILL (Mycologia **4**:163–169. *pl. 68.* 1912) under the title "Illustrations of fungi XI" describes and illustrates several species, 4 of which are new to science. The same author (*ibid.* 205–217) begins a series of articles on the "Agaricaceae of the Pacific Coast"; in the first article 12 new species are characterized.—C. R. ORTON (*ibid.* 194–204. *pls. 70, 71.* 1912) in a paper on "Correlation between certain species of *Puccinia* and *Uromyces*" describes a new fungus (*Puccinia uniporula*). The type was found on *Carex pubescens* Muhl., collected at London, Canada.—L. QUEHL (Monatsschr. für Kakteenk. **22**:102–105. 1912) describes and illustrates a new species of *Echinocactus* (*E. violaciflorus*) from Mexico.—C. REA and H. C. HAWLEY (Proc. Roy. Ir. Acad. **31**: part 13. pp. 1–26. *pl. 1.* 1912) in a report on the fungi of Clare Island, have published a new genus (*Candelspora*); the fungus was found on leaves of *Ilex aquifolia*.—A. REHDER (Rhodora **14**:97–102. 1912) records a new *Rhododendron* (*R. carolinianum*) from North Carolina and a hitherto undescribed form (*R. minus* f. *Harbisonii*) from Georgia.—L. W. RIDDLE (Mycologia **4**:125–140. 1912) enumerates 113 species of lichens, collected in Jamaica by the late Professor CLARA EATON CUMMINGS; the paper includes several new combinations and 11 species new to science.—S. SCHÖNLAND (Rec. Alb. Mus. **2**:251–253. *pl. 12.* 1912) describes and illustrates a new genus (*Neopatersonia*) of the Liliaceae from the region of Port Elizabeth, South Africa.—R. SCHLECHTER (Rep. Sp. Nov. **10**:480–486. 1912) has published 8 new species of orchids from Central America. The same author (Orchis **6**:63–69. *pls. 12, 13.* 1912) in an article entitled "Neue und seltene Garten-Orchideen" describes several novelties, including a new orchid (*Stelis Hennisiana*) native of Colombia.—O. E. SCHULZ (Bot. Jahrb. **46**:613–628. 1912) presents a revision of the genus *Clibadium*, recognizing 19 species, 3 being new to science.—F. J. SEAVER (Mycologia **4**:115–124. *pls. 66, 67.* 1912) publishes the results of a taxonomic study of the genus *Lasiosphaeria*, recognizing 10 species of which 2 are characterized as new.—E. E. SHERFF (Rhodora **14**:164. 1912) records a new variety of *Rudbeckia* (*R. subtomentosa* var. *Craigii*) from Missouri.—S. A. SKAN (Bot. Mag. *t. 8436.* 1912) describes and illustrates a new *Calceolaria* (*C. Forgetii*) from Peru.—M. SLOSSON (Bull. Torr. Bot. Club **39**:285–288. *pl. 23.* 1912) has published two new ferns from tropical America.—W. W. SMITH and G. H. CAVE (Rec. Bot. Surv. Ind. **4**:141–260. 1911) under the title "The vegetation of the Zemu and Llonakh valleys of Sikkim" enumerate somewhat over 1000 species of plants from the Selaginellaceae to the Compositae and include a new genus, namely *Parajaeschkea*, referred to the Gentianaceae.—W. W. SMITH (*ibid.* 273–282) in an article entitled "Some additions to the flora of Burma" describes several species new to science and proposes a new genus (*Craibiodendron*) of the Ericaceae.—A. T. SPEARE

(Phytopathology **2**:135–137. *pl. 12.* 1912) describes and illustrates a new fungus (*Gibellula suffulta*), found on an unidentified species of spider at Wiamea, Hawaii.—P. C. STANDLEY (Proc. Biol. Soc. Wash. **25**:119, 120. 1912) proposes a new genus (*Wootonella*), based on *Ximenesia encelioides* var. *nana* Gray.—F. STEPHANI (Sp. Hep. **4**:801–824. 1912) has issued title-page and index to volume 4, and (*ibid* **5**:1–176) continues the record of species, many of which are new to science.—H. and P. SYDOW (Leafl. Philipp. Bot. **5**:1133–1147. 1912) have published 24 new species of fungi from the Island of Palawan, P.I., and include the following new genera: *Nematothecium* of the Perisporiaceae and *Discosiella*, a genus related to *Discosia*.—F. THEISSEN (Beih. Bot. Centralbl. **29**:45–73. 1912) under the title "Zur Revision der Gattung *Dimerosporium*" characterizes a new genus (*Dichothrix*) of the Eurotiaceae.—W. WEINGART (Monatsschr. für Kakteenk. **22**:83, 84. 1912) has described a new species of *Echinocereus* (*E. Weinbergii*) introduced into cultivation from North America, and (*ibid.* 106–109) a new species of *Cereus* (*C. Vaupelii*) from Haiti.—H. F. WERNHAM (Journ. Bot. **50**:156–164. 1912) presents a revision of the genus *Bertiera*, recognizing 33 species of which 5 are new to science.—K. M. WIEGAND (Rhodora **14**:117–161. *pls. 95, 96.* 1912) presents an interesting and thorough revision of the genus *Amelanchier* in eastern North America, recognizing 8 species of which 3 are characterized as new. The revision is of particular value on account of a clear key to the species and full citation of exsiccatae.—W. ZEH (Notizblatt **5**:268–273. 1912) has described several new species in the genus *Liagora* and includes one from California, one from Guadeloupe, and one from Brazil.—J. M. GREENMAN.

Periodicity of tropical vegetation.—On account of the abundance of its data, VOLKENS[9] report on the results of his observations in Java, 1901–1902, is perhaps the most valuable of a series of recent articles upon the problems and conditions of foliar periodicity in the tropics. Exact records of the behavior of individual trees of over 100 species growing in the Buitenzorg Gardens show almost all possible variations of foliage conditions, from trees regularly deciduous once or twice a year, through evergreens with marked periodicity, to others with uniform foliage gradually renewed throughout the entire year. Attention is directed to the marked individuality not only of species and of individual trees of the same species, but also of individuals of different ages, and even of different branches of the same tree. VOLKENS shows that a moderate amount of climatic periodicity exists at Buitenzorg, especially in precipitation, relative humidity, and insolation; also that a large majority of the trees show a definite foliar periodicity, but concludes that no coincidence or causal connection can be established between the two classes of phenomena. He rejects as most improbable the influence of the salt content

[9] VOLKENS, G., Laubfall und Lauberneuerung in den Tropen. 8vo, pp. 142. Berlin: Gebrüder Borntraeger. 1912. *M* 3.80.

of the soil water, and abandons the hypothesis that leaf-fall is due to an excess of stored food checking the activity of the leaf by preventing the removal of the products of photosynthesis. He finds the final cause of leaf-removal and leaf-renewal in the inherited, internal, unknown attributes of the protoplasm. He confesses that all his efforts to arrive at any explanation of these internal causes have been fruitless. External factors modify the action of these primary causes, but in a comparatively uniform climate like that of Java the modification is slight.

VOLKENS also reviews the work of WRIGHT[10] and others upon deciduous trees, and that of SMITH[11] which affords some data for certain evergreen species in Ceylon, and finds them in accord with his own observations.

In attempts to interrupt the regularity of leaf-fall, DINGLER[12] in Ceylon and KLEBS[13] in Java removed all the leaves from individuals of various species a few months before their regular time of shedding, and obtained a prompt renewal of the foliage and its persistence throughout the period in which they were usually leafless. DINGLER sees in this a proof of the efficiency of the inner cause in disregarding external conditions, while KLEBS, on the contrary, regards it as a response to external factors proving that a rest period is not required. Both these investigators have studied the behavior of deciduous European trees in the tropics, DINGLER[14] at a mountain station in Ceylon, and KLEBS at Tjibodas, Java, and found the usual European habit largely abandoned, new foliage and flowers being often produced twice in the year, while many species never became entirely leafless. Several resembled tropical species in having at one and the same time branches in full foliage, with bursting buds and in leafless condition, respectively. From these and many other experimental studies KLEBS reaches the general conclusion that periodicity of plant life is conditioned by periodicity of external factors.

In a more recent paper[15] KLEBS takes exception to VOLKENS' statement that tropical vegetation in Java is mainly periodic, claiming that when all the constituents of the forests about Buitenzorg and Tjibodas are considered, there

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[10] WRIGHT, H., Foliar periodicity of endemic and indigenous trees in Ceylon. Ann. Roy. Bot. Gard. Peradeniya **2**:415–516. 1905.

[11] SMITH, A. M., On the internal temperature of leaves in tropical insolation; also observations on the periodicity of the appearance of young colored leaves of trees growing in Peradeniya. Ann. Roy. Gard. Peradeniya **4**:229–298. 1909.

[12] DINGLER, H., Versuche über die Periodizität einiger Holzgewächse in den Tropen. Sitz. K. Bay. Akad. Wiss. München. pp. 127–143. 1911.

[13] KLEBS, G., Über die Rhythmik in der Entwicklung der Pflanzen. Sitz. Heidelberger Akad. Wiss. Abh. 23. 1911.

[14] DINGLER, H., Über Periodizität sommergünen Bäume Mitteleuropas in Gebirgsklima Ceylons. Sitz. K. Bay. Akad. Wiss. München. pp. 217–247. 1911.

[15] KLEBS, G., Über die periodischen Erscheinungen tropischer Pflanzen. Biol. Centralbl. **32**:257–285. 1912.

is comparatively little periodicity. By a series of experimental cultures, carried on partly in Java and partly in the greenhouses at Heidelberg with tropical trees of periodic habit, he has succeeded, by varying the fertility of the soil, in obtaining varying responses from the same species and in greatly prolonging the period of continuous growth. This leads him to conclude that periodicity may be conditioned by the supply of food materials. He also calls attention to the fact that in attempts to relate periodicity to external factors only the more obvious climatic conditions have been considered, and that our data consist largely of comparatively crude observations. More exact studies upon an experimental basis are required. He concludes that the idea of a general primary rhythm in tropical plants, as advanced by SCHIMPER and now supported by VOLKENS and others, is contradicted by one series of facts, is rendered doubtful by other facts, and is supported only by such observations as up to the present time it has been impossible to subject to a searching physiological examination.—GEO. D. FULLER.

Paleobotanical notes.—WHERRY[16] has described three types of fossil wood from the Trias of Pennyslvania. The first, *Araucarioxylon virginianum* Knowlton, has been found previously in North Carolina, Virginia, and Connecticut. The second, *A. vanartsdaleni*, is separated as a new species on the lower medullary rays and predominance of uniserial pitting of the tracheids. The third is referred to the genus *Brachyoxylon* Hollick and Jeffrey, under the name of *B. pennsylvanicum*, because the pits, when uniserial, are usually scattered and circular, and when biseriate are either "distant and sub-opposite" or "alternate and hexagonal." This identification seems hardly justified, for the pits in *Brachyoxylon*, when double, are always alternate and closely compressed. Moreover, the most important diagnostic feature of that genus is the formation of traumatic resin canals. Without this reaction, WHERRY cannot properly refer his specimen to the genus *Brachyoxylon*.

In a second paper,[17] WHERRY discusses the evidence supporting the suggestion that the so-called "New Red" may represent deposits from the Lower Carboniferous to the Jurassic, and concludes that while there are no grounds for referring any of these beds to the Paleozoic, the absence of distinctive fossils except those of the Keuper type leaves it an open question whether there may not be also Bunter below and Jurassic above.—R. S. HOLDEN.

Earliest European angiosperms.—Dr. STOPES has described[18] three new genera from the lower Greensand of England, representing the earliest structurally known European angiosperms. The first, *Aptiana radiata*, has vessels

[16] WHERRY, EDGAR T., Silicified wood from the Triassic of Pennsylvania.

[17] ———, Age and correlation of the "New Red" or Newark Group of Pennsylvania.

[18] STOPES, MARIE C., Petrifactions of the earliest European angiosperms. Phil. Trans. Roy. Soc. London B **203**:75–100. *pls. 6–8.* 1912.

with scalariform end walls, copiously pitted fiber tracheids, both uniseriate and multiseriate rays, and very few or possibly no wood parenchyma cells. The vessels and tracheids are of an undoubtedly primitive character, but the rays, according to recent investigations, represent a high state of development. The second, *Woburnia porosa*, has multiseriate rays, and parenchyma grouped around the vessels, as it is in such high families as the Leguminoseae, Ulmaceae, Oleaceae, etc. The third, *Sabulia Scottii*, is imperfectly preserved, but shows mainly uniseriate rays. Whether they represent the primitive condition, like certain species of *Alnus*, or are reduced, like *Castanea*, *Salix*, *Populus*, etc., it is impossible to say. Dr. STOPES seems to think that the antiquity of these specimens indicates that theirs is necessarily the primitive type of angiospermous wood structure. It is significant, however, that in formations considerably older than the Lower Greensand, there are abundant impressions of the Cupuliferae, which comparative anatomy has shown to represent the really primitive conditions.—R. S. HOLDEN.

A new cretaceous palm.—STEVENS[19] has described a new palm from the Upper Cretaceous of New Jersey, the fossil having been found on the beach at Seabright, not far from Sandy Hook. The details are well worked out and illustrated, and the name assigned is *Palmoxylon anchorus*. It seems that petrified stems of palms are not so rare as has been supposed, and the author thinks it probable that palms occur abundantly from the Upper Cretaceous on, both on the coastal plain and in the formations of the continental interior.—J. M. C.

Haustorium of Striga.—Miss STEPHENS[20] has investigated the remarkable haustorium of *Striga lutea*, a South African annual growing as a root parasite on native grasses and on maize. The haustoria arise exogenously from the many adventitious roots, and when one encounters a root of the maize it bores its way into the host by means of a ferment, a line of tracheids is formed down the center of the haustorium, and vascular connections are established with the host.—J. M. C.

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[19] STEVENS, N. E., A palm from the Upper Cretaceous of New Jersey. Amer. Jour. Sci. **34**:421–436. *figs. 24.* 1912.

[20] STEPHENS, EDITH L., The structure and development of the haustorium of *Striga lutea*. Ann. Botany **26**:1067–1076. *pl. 93.* 1912.

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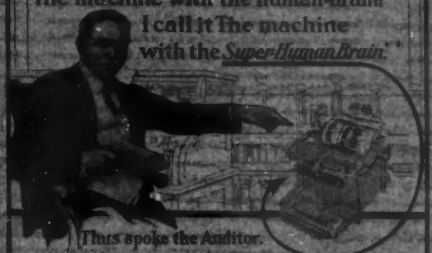

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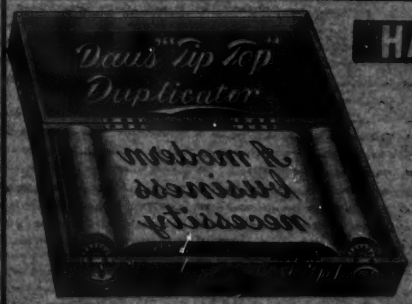

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